



## ATLAS Anti- MUMPS IgM

Indirect Enzyme Immunoassay for the detection of IgM antibodies to MUMPS virus (Parotitis virus).

### 1. INTENDED USE

Indirect enzyme immunoassay for the detection of IgM antibodies to Mumps (Parotitis) virus in human serum and plasma. The enzyme immunoassay is intended for testing individual specimens, not pooled specimens. In vitro diagnosticum, only to be used for in vitro diagnostic purposes by correspondingly educated laboratory personnel. The test can be processed manually or automatically.

### 2. DIAGNOSTIC RELEVANCE, DISEASE AND RECOMMENDED LITERATURE

Besides detection of the infectious agent, the detection of antibodies to Mumps virus significantly contributes to the serological diagnosis of these infections. Laboratory diagnosis of Mumps virus relies on detection of the infectious agent (culture, antigen detection, PCR or LCR) and also on serology (antibody detection by different methods: agglutination, immunofluorescence, complement fixation and ELISA).

Mumps is a systemic illness caused by the Mumps virus, parotitis virus that belongs to the paramyxovirus family which comprises Measles virus, Parainfluenza and Respiratory syncytial virus as human pathogens.

Since the introduction of the vaccine incidence has declined effectively where applied.

Up to 90% of infections at the age of 10 - 14 age are associated with symptoms whilst almost all infections are subclinical beyond 60 years of age.

The mumps virus is transmitted by respiratory droplets. It has an incubation period of 14-25 days after which time prodromal symptoms occur consisting of headache, malaise, myalgia and low grade fever occurs and last anywhere from 1-5 days before the onset of parotid enlargement. The most common clinical presentation is parotitis, but asymptomatic infections may also occur.

Systemic complications may occur (meningitis, encephalitis, hearing loss, orchitis and oophoritis, pancreatitis, arthralgia, Myocarditis, transient renal dysfunction, insulin dependent diabetes and thyroiditis)

Pregnant women contracting mumps during her pregnancy have increased risk for abortion. This is thought to be due to hormonal imbalances caused by virus infection. There is generally no risk of congenital malformation.

Infections with Mumps virus induce neutralizing antibodies and IgM and/or IgA and IgG antibodies appear early and sequentially after infection, .

**Infection:** Mumps virus is usually acquired through the upper respiratory tract by the inhalation of airborne respiratory droplets from an infected host or by direct contact. It has an incubation period of 14-25 days .The virus quickly spreads to the local lymphoid tissue where the primary viraemia occurs, after which the virus spreads to distant sites in the body, reaching different target organs, where a secondary viremia may occur. .

**Virus:** A member of the paramyxovirus family, closely related to parainfluenza viruses, ss RNA, enveloped virus of helical symmetry. Single RNA molecule of 16 - 18 kbp, in a helical nucleocapsid in association with the nucleoprotein (NP)

M protein forms the structure which underlies the viral envelope. The HN (haemagglutination and neuraminidase protein) and the F (fusion) protein form the spikes present on the lipid bilayer envelope, only one serotype of the virus exist s, significant antigenic cross-reaction occurs with other members of the paramyxovirus genus.

### 3. PRINCIPLE OF THE METHOD

Human IgM antibodies against MUMPS virus., if present in the specimen, bind to immobilized MUMPS virus antigens on the surface of the wells of the microtiterplate, the human antibodies bound are then detected by specific anti-human-IgM antibodies labelled to horse radish peroxidase and subsequently revealed by the substrate/chromogen colour reaction. After stopping the colour reaction the initially blue colour turns yellow and the intensity of this yellow colour is measured photometrically (extinction, absorbance, optical density (O.D.)).The intensity of the colour reaction is proportional to the corresponding antibody content

1	Microplate strips , antigen coated (12 x 8 break apart wells)	1	bags
2	Negative Control, human, ready to use (light green)	1	1.5 ml
3	Differential Control, human, ready to use (orange)	1	3 ml
4	Positive Control, human, ready to use (red)	1	1.5 ml
5	Diluent buffer (blue)	2	20 ml
6	Additive for diluent buffer	2	2 ml
7	Anti-human IgM Conjugate, ready to use (yellow)	1	12 ml
8	Substrate buffer with Substrate	1	15 ml
9	Chromogen-concentrate (21x conc TMB in DMSO, corrosive)	1	0.75 ml

10	Washing solution concentrate (25x conc)	2	20 ml
11	Stopping solution (sulfuric acid, 1% V/V)	1	15 ml
12	Package insert		

## 5. MATERIAL REQUIRED BUT NOT PROVIDED

Deionized water, graduated cylinders 1000 ml, 500 ml, 250 ml, 100 ml. Pipettes with a fixed or variable volume of 10, 100, 200 and 1000 microliters. 8-channel pipettes with variable volume of 100 or 200 microliters.

Additional vials (10 ml, 20 ml) for making the ready to use solutions.

Tubes with low protein absorption (e.g. polypropylene, polyethylene or glass) for making sample predilutions if necessary. Incubator 37 °C +/- 1 °C (dry incubator, make sure to correctly seal the wells with the adhesive foil to prevent evaporation which may lead to erroneous results). Timer.

Washing device: using manual or automatic washing devices optimise settings according to the manufacturer's instructions for use, so that the validation criteria of the test are fulfilled.

Microtiterplate reader having a 450 nm filter, reading at the measuring wavelength (450 nm) and using a reference wavelength a filter for 620 to 650 nm is recommended.

## 6. SAFETY MEASURES: Warnings, Precautions, Disposal

**6.1 GLP-RULES** should always be followed (GLP: Good Laboratory Practice).

**6.2** The test kit is only to be used for in vitro diagnostic purposes and by professional staff only.

**6.3** The use of protective laboratory clothes, protective hand gloves and also protective glasses during the actual manual procedure is recommended. Do not pipette by mouth.

**6.4** All tested samples should be regarded as potentially infectious and should be handled accordingly. The controls have been derived from donations which have been tested for anti-HIV 1+2, anti-HCV and HBsAg on a single donor basis and have been found non reactive. Nevertheless they should also be handled as potentially infectious.

Do not use heat inactivated test specimens.

**6.5** Material of bovine origin used as ingredients in reagents, originate from countries known to be BSE-free at the time of purchase.

**6.6** The controls and the additive for the diluent buffer contain < 0.1 % sodium azide and max. 0.05 % thiomerosal as preservatives. The dilution buffer contains max. 0.05 % thiomerosal as preservative.

**6.7** Precautions to be considered using in vitro diagnostic devices containing sodium azide as preservative:

Sodium azide is poisonous, swallowing and contact with skin, eyes and mucous tissue is to be avoided. Sodium azide generates explosive azides with heavy metals like copper or lead. Disposing sodium azide-containing waste solutions, always rinse with enough water.

**6.8** Precautions to be considered using in vitro diagnostic devices containing thiomerosal as preservative:

Thiomerosal is poisonous, swallowing and contact with skin, eyes and mucous tissue should be avoided. Although thiomerosal is also used in some vaccines as preservative in comparable concentrations, in vitro reagents containing thiomerosal should be handled cautiously.

**6.9** Substrate buffer with substrate (No. 8), Chromogen concentrate (No. 9) and stopping solution (No. 11) contain irritant and corrosive substances and should be handled cautiously. If contact with skin, eyes or mucous tissue occurs immediately rinse with enough water and consult a physician.

**6.10** All waste solutions should be collected in adequate vessels containing disinfectants capable of inactivating human pathogenic viruses. Follow the corresponding manufacturer's instructions for use.

**6.11** Disposal: follow the locally ruling safety and disposal laws and regulations for disposal.

## 7. LIMITATIONS AND CAUSES OF ERROR

-It is to be considered that under certain specific laboratory working conditions adjustment of alternative incubation periods may be necessary.

- If reagents are used too cool before reaching room temperature (20...25 °C), a weaker colour development will occur at the end of the test run. On the other hand, if room temperature is high (appr. 30 °C or higher), a stronger colour development will occur at the end of the test run. Under these circumstances the validation criteria of the test run may not be achieved.

-Periodically check functionality of pipettes and instruments used.

-The reagents of the test kit are not to be used after its expiry date.

-Do not use heat inactivated specimens. Avoid testing contaminated samples, strong hemolytic, icteric or lipemic samples, since erroneous results may be obtained.

-To ensure the performance of the test kit storage conditions and stability of the opened and diluted reagents must be strictly respected as depicted under storage and stability.

-Reagent No. 1, antigen coated wells, No. 2, negative control, No. 3, differential control, No. 4, positive control and No. 7, conjugate solution are lot specific and are not allowed to be used together with corresponding reagents from another lot.

-Reagent No. 5, diluent buffer, No. 6, additive for diluent buffer, No. 8 substrate buffer with substrate, No. 9, chromogen concentrate, No. 10, washing solution and No. 11 stopping solution are not lot specific and may be used, if necessary and respecting the corresponding expiry date, in a test run together with corresponding lot specific reagents (Nr. 1, Nr. 2, Nr. 3, Nr. 4 und Nr. 7) from another lot.

-Avoid cross contaminations during manipulations.

-Never use the same vessel for the ready to use conjugate dilution and the ready to use substrate/chromogen solution.

-Since TMB turns blue coloured upon oxidation, any contact of the reagents No. 8, No. 9 and No. 11 with heavy metals should be avoided.

Also protect TMB solutions from direct light exposure.

## 8. STORAGE AND STABILITY

DESCRIPTION	STORAGE	STABILITY	COMMENTS
Closed components of the test kit	2...8 °C	until expiry date	
Opened Microplate strips	2...8 °C	6 weeks	keep storage bag tightly closed avoiding high humidity
Opened components No. 2, 3, 4, 5, 6, 7	2...8 °C	12 weeks	avoid Temperature stress and contamination
Opened substrate buffer (No.8)	2...8 °C	12 weeks	avoid direct exposure to light
Opened TMB/Chromogen solution 21x conc (No.9)	2...8 °C	12 weeks	avoid direct exposure to light
Specimen diluent (No.5 + No.6), ready to use	2...8 °C	12 weeks	prepare only the necessary volume and avoid contamination
TMB-Chromogen/Substrate solution (No.8 + No.9), ready to use	2...8 °C	max. 24 h	prepare only the necessary volume and avoid direct exposure to light
Washing solution, ready to use	2...8 °C	12 weeks	use only a clear solution
	20...25 °C	max. 2 weeks	use only a clear solution
Stopping solution	2...8 °C	until expiry date	

## 9. SAMPLE COLLECTION AND HANDLING

Plasma or serum collected by venipuncture should be tested within 2 days if stored at 2...8 °C after reception. Prolonged storage should be done at -20 °C or lower. Avoid repeated thawing and freezing. Samples showing particles should be centrifuged prior to be processed, to avoid possible erroneous results. Contamination should be avoided, since contaminated samples may also lead to erroneous results. Handle all samples as potentially infectious.

## 10. PREPARATION OF REAGENTS AND TESTSAMPLLES

-All necessary reagents must reach room temperature (20...25 °C) prior to be used.

- If reagents are used too cool before reaching room temperature (20...25 °C), a weaker colour development will occur at the end of the test run. On the other hand, if room temperature is high (appr. 30 °C or higher), a stronger colour development will occur at the end of the test run. Under these circumstances the validation criteria of the test run may not be achieved, and corrective measures may be necessary (prolonging or shortening the incubation period)

Sample diluent (No. 5 + No. 6): 2 ml additive (No. 6) are added to 20 ml diluent buffer (No. 5). Bring only the necessary volume of the ready to use specimen diluent to room temperature.

Washing solution, ready to use: The concentrated washing solution (No. 10) is diluted 1 in 25 with deionised water (20 ml concentrate + 480 ml deionised water), use only clear solutions.

Microplate strips and wells: Take the necessary amount of strips or wells from the bag after they have reached room temperature. Place the required strips or wells firmly in the frame, make sure they are evenly arrayed in the frame. If required fill the empty plate positions with empty wells or strips (not antigen coated) according to the pipetting or washing device used to avoid overflow of fluid during pipetting or washing steps of the test run.

Not required strips or wells (antigen coated) must be transferred into the storage bag, well sealed, avoiding humidity and reset for storage at 2...8 °C.

Specimens: Specimens are tested at a 1/21 dilution. Although testing of other body fluids than serum or plasma is possible, specific adjustment of the conditions is needed.

Conjugate: always prepare only the amount of conjugate needed plus =< 0.1 ml.

Chromogen-Substrate solution: always prepare only the necessary amount of chromogen-substrate solution plus =<0.1 ml. As an example: to 1 ml substrate buffer 0.050 ml TMB-Chromogen concentrate is added (1/21 dilution).

## 11. TESTPROCESSING WITH AUTOMATIC DEVICES

Test processing with automatic devices may be carried out according to the assay definition programs of the automatic device in use ( e.g.: BEP<sup>®</sup> 2000, EtiMax3000, Evolis, Quickstep among others)..

The assay definition program allows the bar code identification of the reagents and of the specimens and their sequential process assignment for the entire process of the test.

After defining the jobs to be done a list of the corresponding reagents needed is generated .including the necessary reagent volumes and their corresponding containers (Specimen diluent, controls, conjugate, TMB-Chromogen/Substrate solution, stop solution and wash solution).

For each single reagent needed the minimal calculated quantities have to be present in the corresponding amounts and in the corresponding bar coded vials to be processed.

The reagent vials prepared are placed in the corresponding reagents' rack for processing.

The racks containing the specimens and the racks containing the reagents can then be introduced in the processing area. During introduction of the racks reading is effected and the position of each reagent and specimen registered.

After verifying the necessary amounts of reagents the plate to be processed is requested (it is possible to align a variety of different tests, the only requirement is that the different assays to be processed must have all the same single incubation periods for each incubation).

Before inserting the plate in the plate holder make sure that besides the wells needed to be processed in their corresponding positions, empty positions are filled with empty wells to prevent overflow of washing solution in the washing chamber.

After inserting the plate it is brought to the pipetting area and the assay/assays job/jobs is/are started.

The job is processed in the following way:

1. Dispensing specimen diluent (0.200 ml) in the wells assigned for the specimens .

2. Dispensing specimens in the wells assigned for specimens (0.010 ml in 0.200 ml, 1:21 dilution).
3. Dispensing the controls in the wells assigned for the controls (0.100 ml).
4. Incubation of the specimens and controls: 30 min. +- 1 min at 37 +- 1°C.
5. Washing
6. Dispensing the conjugate (0.100 ml).
7. Incubation with conjugate: 30 min. +- 1 min at 37 +- 1°C.
8. Washing
9. Dispensing the TMB-Chromogen / Substrate solution (1. cycle 0.025 + 2. cycle immediately there after 0.050 ml).
10. Incubation with TMB-Chromogen / Substrate solution.: 15 min. +- 1 min at room temperature (20...25 °C).
11. Stop of the reaction by adding stopping solution ( 0.100 ml).
12. Photometric reading in integrated photometer.
13. Results may be printed out or further transferred online. The corresponding protocols include validated and evaluated results.

## 12. VALIDATION OF THE TEST, CORRECTIVE MEASURES, GENERAL CONSIDERATIONS

### Validation:

Results obtained in absorbance units (extinction units, O.D. units) for the controls are used if the values of the differential control are higher than 0.080 and lower than 1.000 (optimally between 0.200 and 0.600) and the deviation of the values obtained for the differential control falls within +- 20 % of the mean value. Additionally the corresponding index value of the negative control must be < 0.6 and the corresponding index value of the positive control must be > 1.4. These criteria apply to all our systems.

$$\text{Index value of the controls} = \frac{\text{Absorption at 450nm of the corresponding control}}{\text{Mean absorption at 450nm of the differential control}}$$

Example of a validation: mean value of the absorption of the differential control 1. value: 0.280, 2. value: 0.320

controls	O.D.- value 450 nm	Mean value : 0.300	Index
Absorption (O.D. value) of the negative control (Nr. 2).....0.100			0.100 / 0.300 = 0.333
Absorption (O.D. value) of the differential control (Nr. 3) .....0.280			0.280 / 0.300 = 0.933
Absorption (O.D. value) of the differential control (Nr. 3) .....0.320			0.320 / 0.300 = 1.067
Absorption (O.D. value) of the positive control (Nr. 4).....0.600			0.600 / 0.300 = 2.000

**Are the values obtained within the range of the validation criteria, then the test run is valid, and evaluation can be performed. If the validation criteria are not met, then the test is not valid and must be repeated.**

### Corrective measures:

Before repeating the test, the following possible corrective measures should be considered:

1 ) Example 1: obtaining too high an absorbance value e.g. 1.6 for the differential control, a correction factor of 0.5 can be applied to all values and the test may be revalidated. This revalidation of the test run only applies if the criteria for the Index-value of the positive control (>1.4) and for the negative control (<0.6) also apply.

Alternatively if some sample values are above absorbance 3.0 (OVER), a dilution by factor 2 (dispense additionally 0.2 ml stopping solution to each of the stopped wells mix well and then withdraw 0.2 ml from each of them, 1 in 2 dilution) can be performed on all samples to bring 'OVER' values in the measuring range of the photometer (measuring range of the photometer should be from 0 to 3).

2) Example 2: obtaining too low an absorbance value for the differential control (not under 0.060) a factor of 2 may be applied and the test run revalidated. This revalidation of the test run only applies if the criteria for the Index-value of the positive control (>1.4) and for the negative control (<0.6) also apply.

Alternatively performing the next test run the reaction time can be extended from e.g. 10 min to 20 min or to 30 min.

**Should this possible corrective measures not lead to acceptable results, then the test run has to be repeated.**

### General considerations on peroxidase reactivity:

1) That the peroxidase reaction in our systems is initially practically linear with time and starts levelling off slowly after about 10 to 20 min.

2) Therefore after a reaction time of 20 min the absorbance value of a particular sample will be approximately 2x the value after 10 min. reaction time.

3) This means practically that stopping the reaction of a given sample after 10 min. giving an absorbance value of 0.8, stopping after 20 min. will result in an absorbance value of approximately 1.6.

- 4) Due to the fact that the course of the reaction is practically linear during the first 10 to 20 min. for all reactivities, low and high, the proportions of the different reactivities to each other remain the same.
- 5) Incubations at room temperature lead to higher absorbance values at 30...35°C than at 20...25°C during the same time period (approximately 2x higher). According to our test procedure the reaction time for the Chromogen/Substrate incubation is set at 15 min. +/- 5 min. for the manual procedure, this means between 10 and 20 min. reaction time.
- 6) It is to be considered that the reactivity of the conjugate gradually decreases with time, therefore reactivities are set relatively high at the beginning to assure that the validation criteria apply over the entire stability period claimed.
- 7) Due to these facts it is possible to introduce a corrective factor in case that the absorbance values obtained for the differential control surpasses the upper limit value or remains under the lower limit value, not fulfilling the validation criteria, as specified under corrective measures.

### 13. EVALUATION

Evaluation of test results can be performed if the validation criteria apply. Evaluation of the results for each specimen is done after calculating the Index value for each single specimen. Calculation of the index value corresponds to a normalization of the results against the value obtained for the differential control in each single test run and may be assigned as a 'test reference value'. The Index value is obtained by dividing the absorption value (extinction, O.D. value) of each single specimen by the mean value of the differential control.

$$\text{Index} = \frac{\text{Absorption at 450 nm of a specimen}}{\text{Mean Absorption at 450 nm of the differential control}} \quad (\text{Index of a specimen})$$

Index values (Test reference values) higher than 1.00 are scored reactive and indicate a presence of IgM antibodies, Index values lower than 0.90 are scored non reactive and indicate an absence of IgM antibodies. Index values between 0.90 and 1.00 are scored questionable. For weakly reactive results it is recommended to consider a confirmatory test or to request a second specimen 10 to 14 days later to be tested in the same test run with the first specimen.

#### Example of a qualitative evaluation

Qualitative evaluation is done according to the reactivity of the differential control. All specimens giving Index values higher than that of the differential control are considered as reactive and all giving lower Index values are considered as non reactive. The entire Index range may be divided in ranges with increasing reactivity and to these ranges a diagnostic meaning may be assigned. The higher the reactivity the higher the diagnostic meaning.

Mean value of the differential control : 1. value : O.D. 0.280, 2. value : O.D. 0.320, Mean value: O.D. 0.300

Specimen O.D. 450 nm	Index/ Test reference value	Index range	Evaluation Ranges
Spec. No. 1.....0.080	0.080 / 0.300 = 0.266	< 0.900	non reactive
Spec. No. 2.....0.280	0.280 / 0.300 = 0.933	0.900-1.000	border line
Spec. No. 3.....0.350	0.350 / 0.300 = 1.167	1.000-1.500	weakly reactive
Spec. No. 4.....0.500	0.500 / 0.300 = 1.667	1.500-2.000	reactive
Spec. No. 5.....0.700	0.700 / 0.300 = 2.333	2.000-3.000	highly reactive
Spec. No. 6.....1.000	1.000 / 0.300 = 3.333	3.000-5.000	very highly reactive

#### Example of a quantitative evaluation after introduction of relative units

For clinical reports quantitative results in relative units are usually requested to better assess and assign the results obtained. For this purpose the simplest way is to multiply the Index value with a simple factor and assign the new range of values a new range of units. It is to be considered that these relative units are also based on a logarithmic scale.

Example: multiplying the Index values of the specimens in above table by 10 gives the new unit values (logarithmic scale):  
Relationship between O.D.values, Index values and unit values for the above mentioned results

	Spec. No.1	Spec. No.2	Spec. No.3	Spec. No.4	Spec. No.5	Spec. No.6
O.D.values:	0.080	0.280	0.350	0.500	0.700	1.000
Index values:	0.266	0.933	1.167	1.667	2.333	3.333
Units values:	2.66	9.33	11.67	16.67	23.33	33.33

Further mathematical evaluation methods of the results, like using a standard curve (with serum dilutions) as a reference, or with the help of the <one point quantification> are also possible. However it has to be kept in mind that all these additional evaluation methods use one common basic operation: calculating a reference value of the basic reactivity with at least one standard before further mathematical transformation (logarithmic, exponential, polynomial, 4 PL Model, etc.) is done to obtain the corresponding relative units.

The scales of the relative units found are also divided in reactivity ranges with increasing reactivity, that can be related to an increasing probability of a diagnostic indication

In principle however all these evaluation methods operate with the same originally measured values (absorption, extinction, O.D. value) and corresponding differentiating reactivity ranges.

### 14. INTERPRETATION OF RESULTS

The probability to assign a diagnostic significance to a given reactivity increases with increasing absorption value, or increasing Index value or increasing value of relative units.

EXAMPLE:

Specimen O.D. 450 nm	Index	Index Range	Relative Units (e.g.: Index x 10)	Relative Units Range	Evaluation Ranges	Diagnostic Significance
Spec. No. 1.....0.080	0.266	< 0.900	2.66	< 9	non reactive	- - -
Spec. No. 2.....0.280	0.933	0.900-1.000	9.33	9 –10	border line	- - / +
Spec. No. 3.....0.350	1.167	1.000-1.500	11.67	10 –15	weakly reactive	- / + +
Spec. No. 4.....0.500	1.667	1.500-2.000	16.67	15 –20	reactive	+ + +
Spec. No. 5.....0.700	2.333	2.000-3.000	23.33	20 –30	highly reactive	+ + + +
Spec. No. 6.....1.000	3.333	>3.000	33.33	>30	very highly reactive	+ + + + +

The detection of IgM antibodies during the course of an infection generally indicate a current infection.

It is to be considered that in the early stage of a seroconversion (conversion from negative to positive) the results obtained may still fall under the values of the differential control.

Borderline and weakly reactive results should be retested together with an additional sample drawn 10 to 20 days apart. If no differences in reactivity are detected no evidence for a current infection may be assigned, if clear increments in reactivity are detected, support for a current infection may be indicated.

Positive results with very high IgM reactivities correspond with high probability to the acute phase of a current infection.

Since in the early phase of seroconversion IgM antibodies may not yet be detectable, it is recommended to test at least two specimens drawn 10 to 14 days apart to better assess reactivity increments during the course of seroconversion.

Detection of seroconversion or increasing reactivities indicate current or recent infection.

The simultaneous detection of IgA- and a seroconversion for IgM-antibodies very strongly support a current infection.

**Interpretation of serological results should always only be done together with clinical data.**

Testing for IgM-antibodies, rheuma-factor (RF) interferences may generate false positive results; testing for rheuma-factor, and if positive, retesting of positive specimens after RF-absorption is highly recommended.

## 15. EXPECTED RESULTS: REPRODUCIBILITY, PERFORMANCE CHARACTERISTICS

### **REPRODUCIBILITY:**

Reproducibility of the results of the controls and test specimens in our test systems is calculated according to the mean of the index value (MW), the standard deviation (SA) and the variation coefficient (VK).

$$\text{Coefficient of variation (VK)} = \frac{\text{Standard deviation (SA)}}{\text{Mean (MW)}} \times 100$$

Repeated determinations of the same samples (minimum n=4) in our test systems allow to define the following ranges for the coefficient of variation of a given index value:

**Intraassay coefficient of variation** of a given index value of a sample should be less than +- 10 %, and not greater than +- 20 %, should this occur, so it is mandatory to review the test conditions and working techniques.

**Interassay coefficient of variation** of a given index value of a sample should be less than +- 10 %, and not greater than +- 25 %, should this occur, so it is mandatory to review the test conditions and working techniques.

### **PERFORMANCE CHARACTERISTICS:**

Generally the prevalence of IgM antibodies in a population is restricted to the acute phase of infection. After reactivations but also after vaccination IgM antibodies may also be detected.

Expected results within the reactivity frame of our systems correspond to the distribution of positive and negative samples in our blood donor population. Additionally selected samples (when ever possible from different patient populations, seroconversions, vaccination studies, epidemic studies, endemic regions, etc.) are examined to reassess and optimise our systems.

#### **Reference population**

The expected values for IgM antibody reactivity as adjusted between 2 and 6 %, whereas most positive reactivities are restricted to the border line and low positive range and very seldom high reactivities are encountered.

These settings correspond to a specificity of 94 to 98 % in our blood donor population.

#### **Specificity:**

To determine specificity, reactivity inhibition tests with inactivated, homologous infected cell suspensions and also with non infected cell suspensions, of positive reacting samples are performed. The reactivity of specific positive samples in the ELISA test is blocked after pre-incubation with homologous infected cell suspensions, but not after pre-incubation with non infected cell suspensions.

If the reactivity is also blocked after pre-incubation with the non infected cell suspension a non specific reactivity is indicated.

#### **Sensitivity:**

To determine sensitivity selected samples (when ever possible from patient populations, seroconversion paired samples, vaccination studies, epidemic studies, different endemic regions, etc.) are tested to review and optimize the test settings.

**Relative specificity and sensitivity:**

When comparing different ELISA test systems one should always bear in mind, that obtained results very much depend on the composition of the tested sample population and also on the characteristics of the antigen preparation used, therefore these results are only indicative for the population of samples selected for this comparative testing.

**LITERATURE (Links):**

- 1) MUMPS virus <http://virology-online.com/viruses/MUMPS.htm>
- 2) MUMPS Medical encyclopedia <http://www.nlm.nih.gov/medlineplus/ency/article/001557.htm>
- 3) Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections (7th Edition) 1995  
Edited by Edwin H. Lennette, MD, PhD; David A. Lennette, PhD, and Evelyne T. Lennette, PhD
- 4). [Communicable Disease Control and Laboratory Procedures: www.apha.org/media/science.htm](http://www.apha.org/media/science.htm)
- 5) [Clinical Microbiology Procedures Handbook: http://www.asmpress.org/membooks/product.asp?sku=10004m](http://www.asmpress.org/membooks/product.asp?sku=10004m)

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