Read this Instructions For Use carefully before testing.

For in vitro diagnostic use only

MIZUHO MEDY Co., Ltd.

RS virus kit・Human metapneumovirus kit

Quick Chaser® RSV/hMPV

[General precautions]

1) For in vitro diagnostic use only
2) The diagnosis of RS virus infection and Human metapneumovirus infection should be comprehensively made not only by test result of this product, but also in conjunction with the assessment of clinical progress and results of other tests.
3) Procedures which are not described in instruction for use, are not guaranteed.

[Contents]

1) Test plate- 10 tests
   - ・Mouse monoclonal anti-human RS virus antibodies
   - ・Mouse monoclonal anti-human metapneumovirus antibodies
   - ・Colloidal gold conjugated to mouse monoclonal anti-human RS virus antibodies
   - ・Colloidal gold conjugated to mouse monoclonal anti-human metapneumovirus antibodies
2) Extraction reagent solution vial - 10 vials
   Extraction reagent solution is buffer containing detergent.
3) Swab (for nasopharyngeal swab specimen & for nasal aspirate specimen) - 10 pieces
4) Rack (for extraction reagent solution vial) - 1 piece
5) Filter (for extraction reagent solution vial) - 10 pieces
6) Blue cap (for temporary storage of extraction reagent solution vial) - 10 piece
7) Name label for extraction reagent solution vial - 1 sheet

[Intended Use]

For detection of RS virus antigen in nasopharyngeal swab specimen or nasal aspirate specimen
(Aid in the diagnosis of infection by RS virus)
For detection of human metapneumovirus in nasopharyngeal swab specimen or nasal aspirate specimen
(Aid in the diagnosis of infection by human metapneumovirus virus)

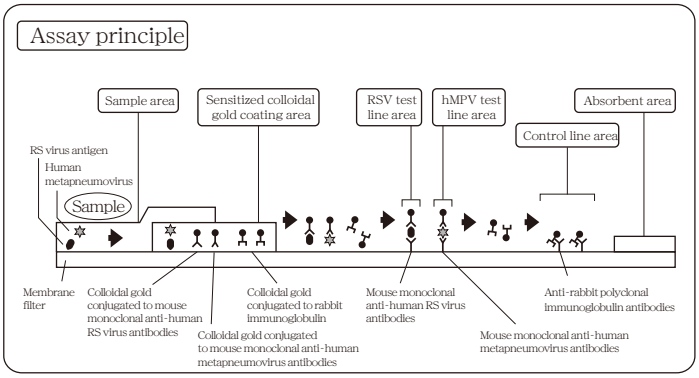
[Principle of the test]

「Quick Chaser® RSV/hMPV」is the in-vitro reagent for detection of RS virus antigen and human metapneumovirus antigen based on Immunochromatographic Assay.

Colloidal gold conjugated to mouse monoclonal anti-human RS virus antibodies, colloidal gold conjugated to mouse monoclonal anti-human metapneumovirus antibodies and colloidal gold conjugated to rabbit immunoglobulin for control line are coated in sensitized colloidal gold coating area in test plate. And mouse monoclonal anti-human RS virus antibodies and mouse monoclonal anti-human metapneumovirus antibodies are immobilized in test line area and anti-rabbit immunoglobulin antibodies for control line are immobilized in control line area.

According to immunochromatographic principle, when samples are added to the sample area, in the presence of RS virus and/or human metapneumovirus, they migrate to the area between sample area and test line area, where respectively reacting with colloidal gold conjugated to mouse monoclonal anti-human RS virus antibodies and colloidal gold conjugated to mouse monoclonal anti-human metapneumovirus antibodies, and react with mouse monoclonal anti-human RS virus antibodies and mouse monoclonal anti-human metapneumovirus antibodies and they are caught in test line area.

As the result, purple- red line is visible. Moreover, purple-red line is also simultaneously visible for catching colloidal gold conjugated to rabbit immunoglobulin by anti-rabbit immunoglobulin antibodies in control line area, regardless of presence of RS virus antigen and human metapneumovirus antigen.



[Procedural precautions]

1) Do not use saliva and sputum as a specimen.
2) Collected specimen should be prepared as sample in accordance with after-mentioned **"Preparation of sample in Test procedure"** and tested as soon as possible.
3) Add fixed volume (3 drops) to the center of sample area from tip of filter about 10mm away from the sample area so as to make droplets. In case of adding other than fixed volume, an accurate reaction may not be performed.
4) Bring test plate and extraction reagent solution to 15～30 ℃ prior to testing.
5) Keep interpretation time inevitably because it causes false-negative and false-positive.
6) Interfering substances and medications
   The following substances and blood did not interfere the performance of this product at the concentration listed below:

   Acetyl salicylate (10 mg/ml)
   Ibuprofen (20 mg/ml)
   Diphenhydramine hydrochloride (5 mg/ml)
   Oxymetazoline hydrochloride (10 mg/ml)
   Dextromethorphan hydrogen bromide (5 mg/ml)
   Phenylphrine hydrochloride (50 mg/ml)
   Cold medicine (concentration of Acetaminophen: 10 mg/ml)
   Nasal drop ①, containing Sodium cromoglicate, Chlorpheniramine monomaleate, Naphozoline hydrochloride (20%)
   Nasal drop ②, containing Ketotifen fumarate (10%)
   Inhalation ①, containing Salbutamol sulphate (20%)
   Inhalation ②, containing Bromhexine hydrochloride (20%)
   Gargle ①, containing Tincture of Myrrh (0.5 %)
   Gargle ②, containing Povidoneiodine (2.0 %)
   Intraoral antiphlogistics, containing Sodium Azulene Sulfonate (10 %)
   Throat candy ①, containing Di-potassium Glycyrrhizinate (20 mg/ml)
   Throat candy ②, containing Nandina Fruit Extract (Dry) (20 mg/ml)
   Throat candy ③, containing Cetylpyridinium chloride (20 mg/ml)
   Blood (1%)

7) Cross reactivity

Cross reactivity were not observed with the following viruses and bacteria.

・Viruses

Influenzavirus A、Influenzavirus B、Adenovirus type 1、Adenovirus type 2、Adenovirus type 3、Adenovirus type 4、Adenovirus type 5、Adenovirus type 6、Adenovirus type 7、Adenovirus type 11、Human Coronavirus、Coxsackie virus A9、Coxsackie virus B5、Human Echovirus 9、Herpes simplex virus type1、Mumps virus、Parainfluenza virus 1、Rhinovirus 8

・Bacteria

Bordetella pertussis、*Candida albicans*、*Haemophilus influenzae*、*Klebsiella pneumoniae*、*Listeria monocytogenes*、*Moraxella catarrhalis*、*Mycoplasma pneumoniae*、*Pseudomonas aeruginosa*、*Serratia marcescens*、*Staphylococcus aureus*、*Staphylococcus epidermidis*、*Streptococcus agalactiae(Group B)*、*Streptococcus mutans*、*Streptococcus pneumoniae*、*Streptococcus pyogenes (Group A)*

[Test procedure]

●Specimen collection and sample preparation

1) Preparation of specimen collection

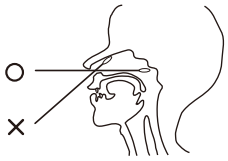
① Swab : Use swab, included in this test kit.

② Extraction reagent solution: Use it without preparation.

2) Specimen collection

① Nasopharyngeal swab specimen:

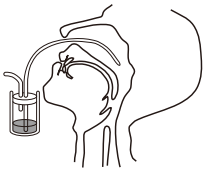
Along inferior nasal conchae (imaging a horizontal plane connecting nostril with external acoustic meatus), insert swab in nasal cavity and rub it on the mucosal surface several times to collect mucous epidermis.



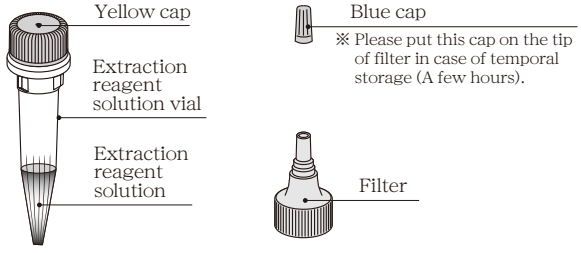
Note) Elastic plastic rod is employed in swab to reduce the burden of patients. However, on the other hand, the tip of swab may not be reached to inflamed region on the wall of nasal cavity or you may not be able to rub the tip on the wall of nasal cavity adequately to collect enough volume of virus antigen, even though the tip of swab is reached to inflamed region on the wall of nasal cavity. Therefore, make sure the tip of swab is reached to the wall of nasal cavity so as to rub the inflamed region at the time of collecting mucous epidermis.

② Nasal aspirate specimen:

Dip spherical tip into low-viscosity liquid part of nasal aspirate specimen in trap. If it is difficult to collect specimen due to high viscosity or low volume of specimen, add 0.5~1mL of saline to specimen, and mix them uniformly. Test can be performed by using the specimen.Be reminded that sensitivity is decreased by dilution of specimen with saline.

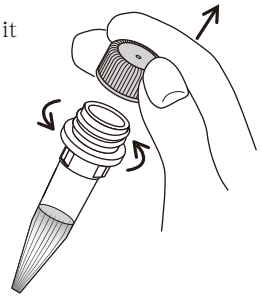


●Details of Extraction reagent solution vial

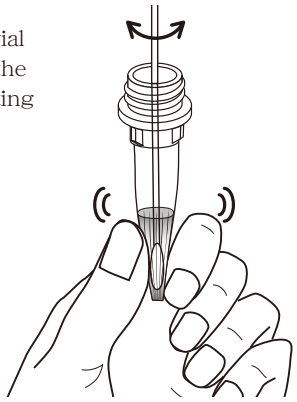


●Preparation of sample

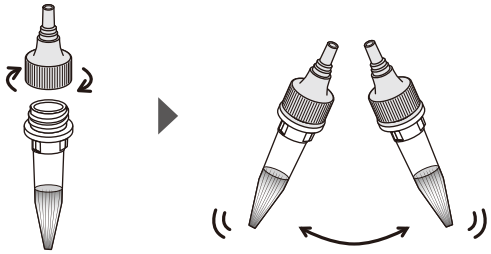
① Loosen yellow cap with turning it counterclockwise.



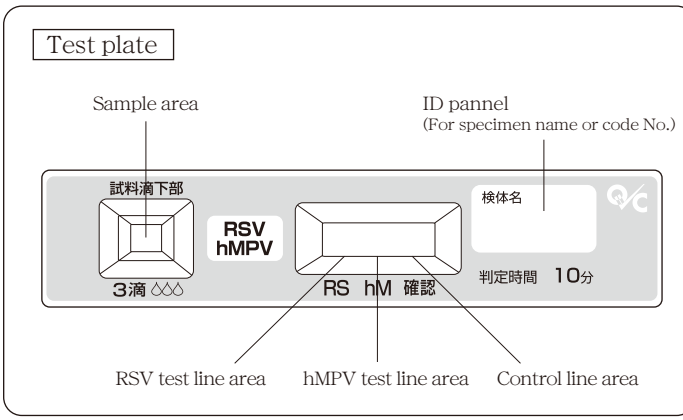
② Insert the spherical tip with specimen, into the bottom of extraction reagent solution vial and press spherical tip from the outside of the vial for extracting specimen and turn the swab clockwise and counterclockwise about five times and rub the spherical tip on the inside wall and bottom of the vial.
Take swab out of the vial with squeezing out liquid from the spherical tip, pressing spherical tip.



③ Install filter and shake the vial several times gently to mix specimen thoroughly. The sample is ready for use.



●Details of test plate



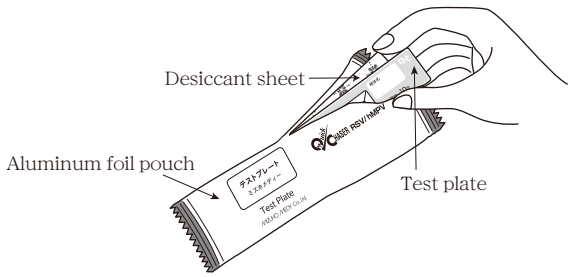
●**Test procedure**

1) Preparation of reagent

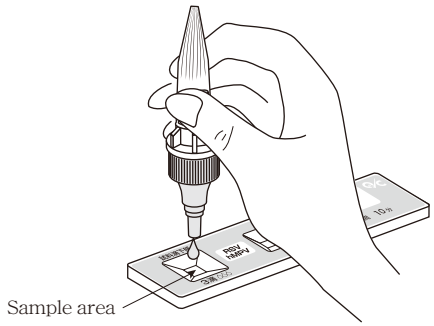
Test plate: No prior preparation required

2) Test procedure

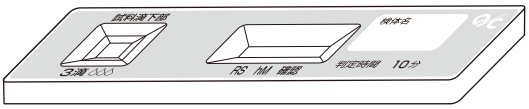
① Remove test plate from aluminum foil pouch.
Discard desiccant sheet.



② Add 3 drops (about 110 μL) of sample vertically to the sample area of test plate from extraction reagent solution vial including prepared sample with avoiding contact of tip of extraction filter with sample area.



③ Leave to react at 15～30℃.
Visually interpret test result by lines in test line area and control line in control line area after 5～10 minutes.



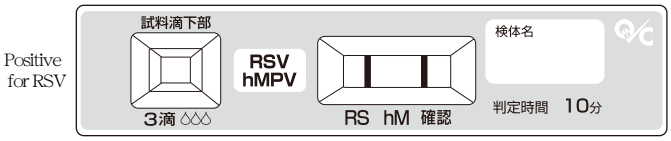
[Interpretation]

Interpret by existence of red-purple lines in test line area for RSV (RS), test line area for hMPV (hM) and control line area.

《Positive》

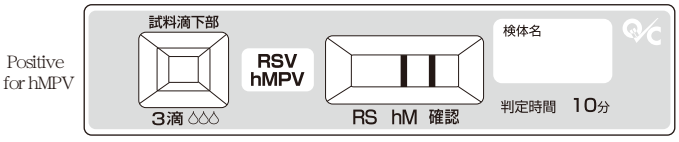
[Positive for RSV]

Test line for RSV and control line appear.



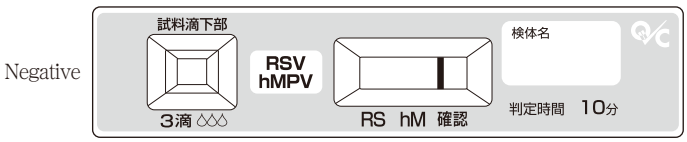
[Positive for hMPV]

Test line for hMPV and control line appear.



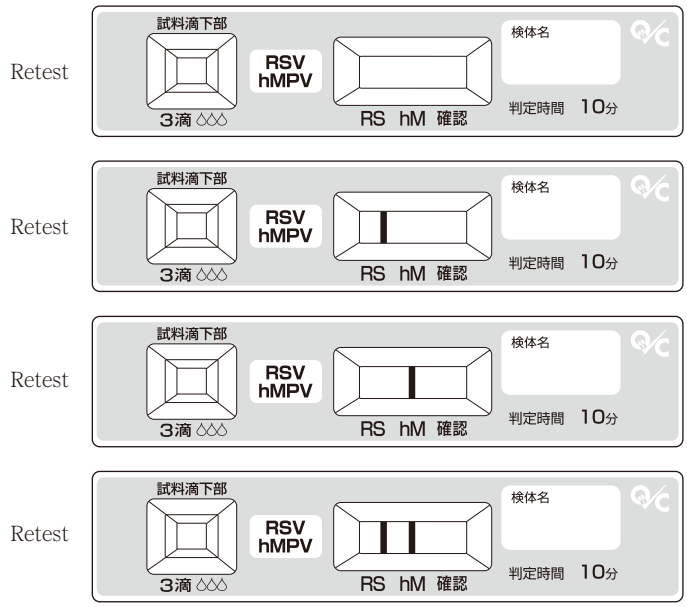
《Negative》

Only control line appears.



《Retest》

If both test lines and control line do not appear or no control line appear, operational mistakes such as the lack of sample are thought. Recheck test procedure and retest with new test plate. If the same result comes out in the retest again, confirm it with other method.



●**Interpretational precautions**

1) In case RSV test line or hMPV test line and control line appear at 5～10 minutes after dropping sample, it can be interpreted as RSV positive or hMPV positive. Negative should be interpreted at 10 minutes after dropping sample. Streak line might appear before 10 minutes temporarily. Do not interpret the temporal streak line as appearance of test line. After 10 minutes, colloidal gold can appear like line due to drying of test plate with time. Therefore, please interpret test results at 10 minutes.

2) This product is used as aid in the diagnosis for infection of RS virus and human metapneumovirus. In case RS virus antigen and human metapneumovirus in specimen are below the detection limit of the test or specimen collection are not enough, test result could be interpreted as negative, even though patients are infected by RS virus and human metapneumovirus. Moreover, factors in specimen could cause non-specific reaction and negative specimen could be interpreted as positive. The definitive diagnosis should be made comprehensively in conjunction with the assessment of clinical progress and results of other tests for confirmation.

3) When lines appear in both of RSV test line area and hMPV test line area, there is a possibility of the superinfection of RS virus and the human metapneumovirus. Collect specimen again and retest it with new test plate just in case. The diagnosis should be made comprehensively in conjunction with the assessment of clinical progress and results of other tests.

[Performance characteristics]

1) Performance

① Sensitivity

・When in-house RS virus positive control Note 1) was tested, RS virus positive result was obtained.

・When in house human metapneumovirus positive control Note 2) was tested, human metapneumo virus positive result was obtained.

② Accuracy

・When in-house RS virus positive control was tested, RS virus positive result was obtained.

・When in-house human metapneumovirus positive control was tested, human metapneumovirus positive result was obtained.

・When in-house negative control Note 3) was tested, negative result was obtained.

③ Reproducibility

・When in-house RS virus positive controls were tested three times simultaneously, RS virus positive results were obtained in all cases.

・When in-house human metapneumovirus positive controls were tested three times simultaneously, human metapneumo virus positive results were obtained in all cases.

・When in-house negative controls were tested three times simultaneously, negative results were obtained in all cases.

Note 1) a RS virus purified antigen are diluted by extraction reagent solution to become the 3.64×10^{9}copies/mL.

Note 2) a human metapneumo virus purified antigen are diluted by extraction reagent solution to become the 9.57×10^{6}copies/mL.

Note 3) Extraction reagent solution

④ Detection limit

RS virus

SubtypeA/A2 strain	9.10×10^{8}copies/mL
SubtypeA/Long strain	2.84×10^{7}copies/mL
SubtypeB/18537 strain	4.55×10^{8}copies/mL
SubtypeB/9320 strain	1.82×10^{9}copies/mL

Human metapneumovirus

SubtypeA1	2.39×10^{6}copies/mL
SubtypeB1	2.99×10^{5}copies/mL

⑤ Reactivity

It is confirmed that this product reacts with RS virus type A/A2, A/Long, B/CH18537 and B/9320 and human metapneumovirus A1, A2, B1 and B2.

2) Correlations

Comparison with existing products

●Nasopharyngeal swab specimen

<RS virus>

Quick Chaser RSV/hMPV

Other product (1)	Positive	Negative	Total
Positive	55	0	55
Negative	1 ※1	71	72
Total	56	71	127

Positive agreement rate : 100%(55/55)
Negative agreement rate : 98.6%(71/72)
Total agreement rate : 99.2%(126/127)

※1 Negative by other product (1), but positive by Quick Chaser RSV/hMPV
The one sample was positive by RT-PCR.

Quick Chaser RSV/hMPV

Other product (2)	Positive	Negative	Total
Positive	56	0	56
Negative	0	71	71
Total	56	71	127

Positive agreement rate : 100%(56/56)
Negative agreement rate : 100%(71/71)
Total agreement rate : 100%(127/127)

<Human metapneumovirus virus>

Quick Chaser RSV/hMPV

Other product (3)	Positive	Negative	Total
Positive	51	0	51
Negative	3 ※2	61	64
Total	54	61	115

Positive agreement rate : 100%(51/51)
Negative agreement rate : 95.3%(61/64)
Total agreement rate : 97.4%(112/115)

※2 Negative by other product (3), but positive by Quick Chaser RSV/hMPV
The three samples were positive by RT-PCR.

Quick Chaser RSV/hMPV

Other product (4)	Positive	Negative	Total
Positive	51	0	51
Negative	3 ※3	61	64
Total	54	61	115

Positive agreement rate : 100%(51/51)
Negative agreement rate : 95.3%(61/64)
Total agreement rate : 97.4%(112/115)

※3 Negative by other product (4), but positive by Quick Chaser RSV/hMPV
The three samples were positive by RT-PCR.

●Nasal aspirate specimen

<RS virus>

Quick Chaser RSV/hMPV

Other product (1)	Positive	Negative	Total
Positive	65	0	65
Negative	0	53	53
Total	65	53	118

Positive agreement rate : 100%(65/65)
Negative agreement rate : 100%(53/53)
Total agreement rate : 100%(118/118)

Quick Chaser RSV/hMPV

Other product (2)	Positive	Negative	Total
Positive	65	0	65
Negative	0	53	53
Total	65	53	118

Positive agreement rate : 100%(65/65)
Negative agreement rate : 100%(53/53)
Total agreement rate : 100%(118/118)

<Human metapneumovirus virus>

Quick Chaser RSV/hMPV

Other product (3)	Positive	Negative	Total
Positive	52	0	52
Negative	2 ※4	52	54
Total	54	52	106

Positive agreement rate : 100%(52/52)
Negative agreement rate : 96.3%(52/54)
Total agreement rate : 98.1%(104/106)

※4 Negative by other product (3), but positive by Quick Chaser RSV/hMPV
The two samples were positive by RT-PCR.

3) Calibration reference material (Standard material)

RS virus antigen solution (in-house standard)

Human metapneumovirus antigen solution (in-house standard)

[Precautions for use, handling]

1) Precautions for handling (Prevention of danger)

① HIV, HBV, and HCV could be included in specimen. Be careful of handling specimen as potentially infectious material.

② Be careful not to touch sample (specimen) or extraction reagent solution directly to skin or not to get them into eyes, wearing glasses, disposable glove or mask at the time of use.

③ Do not use swab to collect specimen, if it is already put into extraction reagent solution.

④ If specimen (sample) or extraction reagent solution are got into eyes or mouth, flush with a plenty of water as emergency treatment and see a doctor, if necessary.

⑤ Do not use blue cap which belong to kit for transporation or preservation because it does not have seal stregnth.

⑥ Perform the collection of specimen under the guidance of the qualified person.

⑦ Raw material of membrane which is used for test plate, is nitrocellulose. Do not perform test near fire because nitrocellulose is extremely flammable material.

⑧ Regarding aspiration tube with trap for nasal aspirate specimen, use unused and contamination-free one per test not to cause contamination for preventing infection spread and maintaining accuracy of test.

⑨ Wipe off with alcohol for disinfection in case of getting splattered with sample (specimen).

2) Precautions for use

① Do not freeze this product. Store it in accordance with description of instructions for use. Do not use frozen reagents because they could show false results by change of quality.
② Do not use this product beyond expiration date.
③ Do not store extraction reagent solution vial with laying down or inverting.
④ Do not use the extraction reagent solution of other products, using the one included in this kit"
⑤ Immediately use test plate after opening of foil pouch. If it is left for long time after the opening, it could be non-reactive due to getting moistened.
⑥ Do not touch sample area, test line area and control line area by hands directly.
⑦ Do not perform test in the place such as under air conditioner where the dry wind directly blows the surface of test plate, to prevent uneven migration.
⑧ Do not use reagent, accessaries etc. of the product except the purpose of this product.
⑨ Test plate, swab, and extraction reagent solution vial (Filter, green cap and blue cap are included) are intended for single use only.
⑩ Use swab, included in this product.
⑪ Store swab avoiding direct sunlight, high temperature and humidity with being careful about the water wets.
⑫ Do not touch spherical tip of swab before use.
⑬ When swab is taken out of a pouch, do not press spherical tip (sponge) and rod (handle) of the swab over the pouch by hand fingers. Load on the spherical tip may make spherical tip secede or drop spherical tip off from the rod.
⑭ Use swab immediately after opening the pouch.
⑮ If break and/or hole are found on the pouch of swab, do not use it.
⑯ If swab is stained, broken or bent, do not use it.
⑰ Do not bend and curve the rod of swab before collecting specimen.
⑱ Be careful not to break the rod of swab and injure region to be collected (mucous epidermis) by excessive force or pushing too hard at the time of specimen collection.
⑲ Elastic plastic rod is employed in swab to reduce the burden of patients. However, on the other hand, the tip of swab may not be reached to inflamed region on the wall of nasal cavity or you may not be able to rub the tip on the wall of nasal cavity adequately to collect enough volume of virus antigen, even though the tip of swab is reached to inflamed region on the wall of nasal cavity. Therefore, make sure the tip of swab is reached to the wall of nasal cavity so as to rub the inflamed region at the time of collecting mucous epidermis.
⑳ Be careful not to splatter the sample at the time of taking the swab out of vial after preparing sample.
㉑ In case collection volume of specimen is excess, or high-viscosity, membrane filter could be clogged and adequate sample volume could not be dropped. In such cases, collect specimen again and retest it with new test plate.

3) Precautions for waste disposal

① Treat liquid waste and used utensils by any one of following methods because sample (specimen) could contain infectious material such as HIV, HBV, and HCV etc.
   a) Immerse in sodium hypochlorite solution (effective chlorine concentration of 1000ppm) for more than 1 hour.
   b) Immerse in 2% glutaraldehyde solution for more than 1 hour.
   c) Autoclave at 121° C for more than 20 minutes.
② Regarding disposal of reagents and utensils etc. , dispose of them as medical waste, industrial waste or infectious waste in accordance with your waste disposal laws and regulations.

[Storage・Expiry]

・Storage: 1～30℃
・Expiry:24 months (As indicated on package)

[References]

1)Susanne Abels et al. : Journal of Clinical Microbiology, 39(9), 3135～3139(2001)
2)Gulden Yilmaz et al. : Journal of Clinical Microbiology, 37(7), 2390(1999)
3)Gurmukh Ahluwalia et al. : Journal of Clinical Microbiology, 25(5), 763～767(1987)

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