EarlyFound® MMP-1 Rapid Strip Test

For in vitro diagnostic use only

Specimen type saliva
Specimen volume 50 µL

Intended Use

The device is an in vitro diagnostic device that qualitatively detects, by immunochemical techniques, the human Matrix metalloproteinase-1 (MMP-1) in human saliva specimens from individuals. A positive result is defined as a visible signal on the T line of the strip and indicates salivary MMP-1 was detected (C_{50}: 412.5 pg/mL; C_{95}: 487.5 pg/mL). The device is intended for use on individuals 30~90 years as an aid in diagnosis of oral squamous cell carcinoma cell (OSCC) at oral cavity including cheek mucosa, floor of mouth, gum, retromolar area, tongue (excluding base of tongue), and multiple sites at the oral cavity, in conjunction with other laboratory findings, imaging studies, and clinical assessment. The intended user is clinical or laboratory professionals.

Introduction

Human Matrix metalloproteinase-1 (MMP-1) is the earliest-identified MMP family member involved in extracellular matrix (ECM) remodeling and is closely associated with metastasis, angiogenesis, and inflammation in tumorigenesis. Upregulated expression of MMP-1 has been reported in several types of cancer, including oral cancer. Elevated mRNA and protein levels of MMP-1 have been confirmed in both tissue and saliva specimens from oral squamous cell carcinoma (OSCC) patients[1]. The level of salivary MMP-1 was dramatically elevated in patients with OSCC but only slightly increased in patients with Oral Potentially Malignant Disease (OPMD)[2]. Salivary MMP-1 measurement will aid in diagnosis of oral mucosa malignant disorders. Based on clinical trial results, MMP-1 in 327 OSCC saliva was 2717±2577 (mean±SD) pg/mL; in 704 OPMD saliva was 255±634 (mean±SD) pg/mL.

The test result does NOT replace an oral cancer screening or an oral cancer diagnosis. If the level of salivary MMP-1 changes or increases, it may be caused by oral mucosa malignant disorders. The subjects need to go to a doctor for further oral examination such as visual inspection, dental radiology exam, and biopsy for diagnosis. A negative result does NOT rule out the possibility of OSCC.

[1] ref: CEBP 2011, 20(12), 2628; doi: 10.1158/1055-9965.EPI-11-0503.

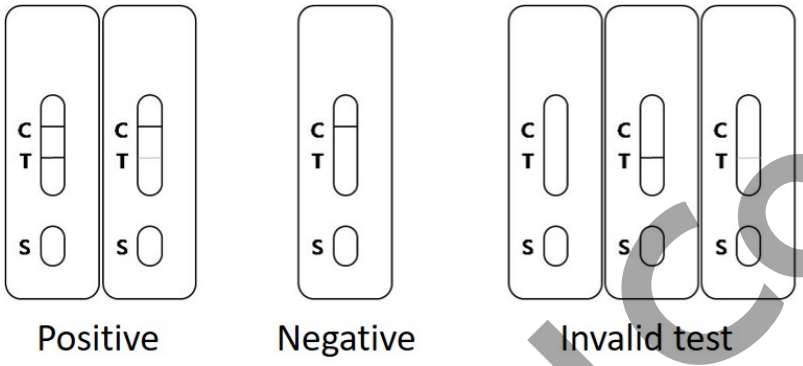
[2] ref: Cancers 2020, 12, 2273; doi: 10.3390/cancers12082273.

Principle of the Assay

MMP-1 Rapid Strip Test is a colloidal-gold based lateral flow test that detects the human MMP-1

protein in saliva specimen. To use the test, a saliva specimen is added to the reaction buffer. Next, saliva mixture is applied to the sample well (S) of the test device. If the sample contains MMP-1 proteins, they will bind to the MMP-1 antibody (Ab) conjugated-colloidal gold to form MMP-1-gold complexes. When these complexes reach the Test line (T) of the strip, they will be retained on the test line by another set of MMP-1 Abs and form a red visible signal. Therefore, the red line signal shown in the Test line (T) area indicates the salivary MMP-1 detected by the test. Additionally, a red line signal should be visible in the Control line (C) area if the test was performed correctly.

Result demonstration:



Product Content

Catalogue number：STBP01R1-30T

No.	PART	DESCRIPTION	Amount
1.	Single test device with seal pouch	Each test device contains a colloidal-gold based lateral flow strip which can recognize the MMP-1 protein from saliva specimen by monoclonal Abs specific for human MMP-1. The test device is sealed in a white pouch with a desiccant inside as a humidity indicator.	Single test*30 pouches
2.	Reaction buffer	To be mixed with saliva specimen before the test. ProClin™ contained.	5 c.c./tube*1
3.	Instructions for Use	Package insert.	*1

NOTE：

1. Please read the instructions for use before use.
2. Perform the test immediately after removing the test device from the sealed pouch to avoid humidity interference.
3. Do NOT use the test device if the desiccant turns pink. Only use the test device when the desiccant is blue.

4. Do NOT reuse the test device.
5. Do NOT mix or substitute reagents with those from other lots or sources.
6. If crystals have formed in the Reaction buffer, mix gently until the crystals have completely dissolved.

Storage and Stability

- ❑ Do NOT freeze the kit.
- ❑ Keep in cool and dry place, avoid heat and long-term sunlight exposure.
- ❑ Shelf-life: 12 months. Store at room temperature.
- ❑ Transport stability: 2 weeks in cold (2~10°C) or in 55°C.
- ❑ In-Use stability of Single test device with seal pouch: 1 hour.

Materials Required but Not Provided

- ❑ Sample collection cup/tube.
- ❑ General laboratory equipment including: vortex mixer, mini-centrifuge, timer, pipettes, pipette tips and microtubes.
- ❑ Disposable gloves, protective clothing and biohazard disposal container.

Precautions

- ❑ Do NOT use the device beyond the expiration date or if its package has been damaged.
- ❑ Read the instructions for use carefully before performing the test.
- ❑ Follow standard Lab procedures and biosafety guidelines for handling and disposal of potentially infectious material. All specimens and reagents should be considered potential hazardous.
- ❑ Some components contain ProClin™ which may cause an allergic skin reaction. Avoid breathing mist and direct contact with human body.
- ❑ Wear protective gloves, clothing, and goggles for eye and face protection during operation. Wash hands thoroughly after handling.

Saliva Specimen Collection and Storage

Subjects should NOT eat food, drink and smoke in 60 minutes before collection. Rinse mouth 5 times with drink water (Never Use Mouthwash), then move tongue in oral cavity to stimulate saliva secretion. Spit out saliva into collection cup/tube until a sufficient amount (at least 3 mL) is collected. Store the crude saliva on ice or at 4°C before centrifugation. Centrifuge the specimen at 3000 x g for 15 minutes to collect supernatant for assay. Perform the assay immediately or aliquot and store the specimen at -70°C (stable for 180 days). Cryopreserved specimen should avoid repeated freeze-thaw cycles.

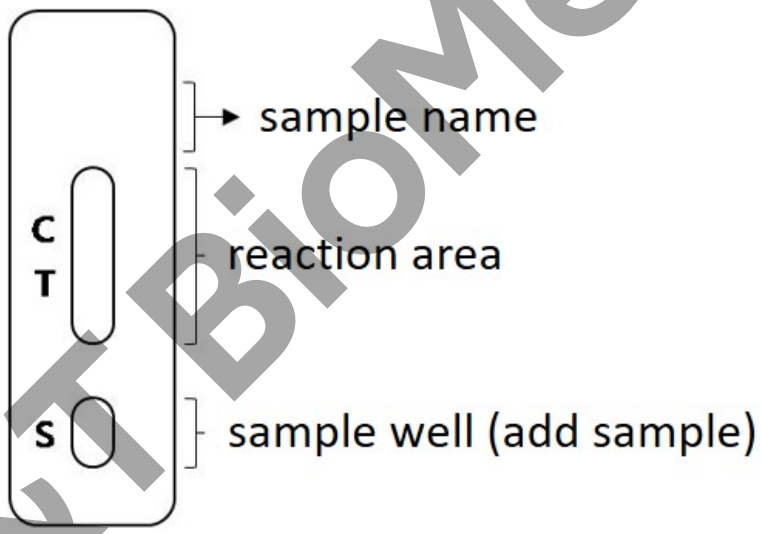
NOTE：

1. Saliva collection should be avoided under bleeding conditions. Do NOT use saliva that contains blood.
2. Rinse mouth with drinking water (Never Use Mouthwash).

Assay Procedure

A replication test is recommended

1. Rest the saliva specimen at room temperature for at least 5 min.
2. The saliva specimen should be centrifuged for 30 sec. with a mini-centrifuge until the suspension debris settles down.
3. Label the specimen name on the top of the microtube and add 50 µL of the reaction buffer into the microtube.
4. Take out the test device from the sealed pouch and keep the test device flat.
   NOTE：Do NOT use the test device if the desiccant turns pink. Only use the test device when the desiccant is blue.
5. Label the specimen name on the top of the test device.
6. Add 50 µL of the saliva specimen into the microtube which contains 50 µL of the reaction buffer and mix well (saliva mixture).
7. Add 100 µL of the saliva mixture into the sample well until the mixture is absorbed by the test device.



8. When the mixture runs into the window of the reaction area, start the timer and wait for 20 minutes. Read the data at 20 minutes and check again at 30 minutes to prevent missing reading of the slow emergence of weak positive signal.
   NOTE：Do not touch the test device and avoid contact with other substances during this period so as not to affect the test results.

9. Three different results may be interpreted from the test device:
   ①If Control (C) line is NOT present, the test fails and is considered Invalid
   ②Positive result: BOTH Control (C) line and Test (T) line are present, this means that salivary MMP-1 is detected.
   ③Negative result: Only Control (C) line is present, this means that salivary MMP-1 is not detected.

Technical Hints

- ❑ When mixing solution, always avoid foaming.
- ❑ Perform the test immediately after removing the test device from the sealed pouch to avoid the humidity interference.
- ❑ Read the test result 20~30 minutes after applying the saliva mixture into the well. Do NOT read the result after 30 minutes since a false positive result may be generated

Limitation of the Procedure

- ❑ The test result does NOT replace an oral cancer screening or an oral cancer diagnosis.
- ❑ If the saliva specimen is so sticky, it may affect the test performance and/or produce invalid results.
- ❑ Do NOT add water if the saliva specimen less than 50 µL as it may occur a false negative result.
- ❑ Do NOT test the saliva specimen contains blood as it may cause a false negative (inaccurate) result.
- ❑ Oral medicine/spray may affect test performance and/or produce invalid results.
- ❑ Variation in saliva collection, processing, and storage may cause an inaccurate result.
- ❑ The device is based on an immunochemical reaction. Patients receiving monoclonal antibody treatment may produce HAMA (human anti-mouse antibody), which may cause an invalid result.

Performance Characteristics

- **Analytical sensitivity**
  C_{50}: 412.5 pg/mL.; C_{95}: 487.5 pg/mL.

- **Precision:**
  Three positive and one negative samples were used. The result among 10 replicates, 3 lots, 5 runs, or 4 operators were 100% consistent.

- **Cross-reactivity:**
  Six common salivary MMPs, including MMP-2, MMP-3, MMP-7, MMP-8, MMP-9 and MMP-13 were tested and shown negative results that indicate no cross-reactivity.

- **Interference:**
  Risk factors of oral cancer: According to the clinical study, risk factors of OSCC including personal habits of betel nut chewing (former or current) and smoking (former or current) showed no interference with the detection of MMP-1[ref].
  *** ref: Cancers 2020, 12, 2273; doi: 10.3390/cancers12082273.**
  Common components in saliva: The MMP-1 levels were only correlated with total protein levels but not levels of IgA or alpha-amylase, suggesting that both IgA and alpha-amylase are not interfering factors for MMP-1.
  Specimen condition: Hemoglobin (2 g/L) contamination in saliva will cause high red background to interfere with T-line signal visibility and cause a false negative result. BE SURE to collect saliva specimens following the instruction "Saliva collection should be avoided under bleeding condition. Do NOT use saliva that contains blood.".
  External source substances: Dietary Substances (including proteins residuals and common drinks), Mouthwash, and Oral medicine/spray (listed below) were added for testing. Some mouthwash and oral medicine caused false results (1/10 of the interfering substances were added) but the interference could be eliminated by additional dilution of interfering substances (1/1000 of the interfering substances were added). In other words, sufficient mouth wash with drink water could decrease external source interference. BE SURE to collect saliva specimens following the instruction "Subjects should NOT eat food, drink and smoke in 60 minutes before collection. Rinse mouth 5 times with drink water (Never Use Mouthwash).".

interfering substances	**1/10 of the interfering substances were added**

Whole milk	No effect
Soy milk, sugar free	No effect
Coffee, sugar free	No effect
Black tea, sugar free	No effect
Juice (100% orange juice)	No effect
Carbonated drink (sugary Coke)	No effect
Parmason Gargle Solution, green	No effect
LISTERINE Total care Mouthwash, crystal violet	No effect
LISTERINE Original Mouthwash, caramel	False negative
YADRAN Mouthwash, blue	No effect
Eudesmol, 0.092% w/v.	No effect
Xylitol, 0.09 g/mL	No effect
BECLOMET NASAL AQUA, including beclomethasone, 1.11 mg/mL.	No effect
CUFFLAM ANTI-INFLAMMATORY SPRAY, including Benzydamine, 1.5 mg/mL.	No effect
BETADINE® Throat Spray, including povidone-iodine, 0.45% w/v.	False positive
TCHHTHUSUI, including Benzocaine, 0.067 gm/mL.	False negative
Togiam, including polycresolsulfonate, 50%.	Invalid result
MUNDISAL GEL, including choline salicylate, 0.02 g/mL.	No effect
Oralog Orabase 1mg/g "Purzer", including triamcinolone acetonide, 0.01 g/mL.	No effect
Koulening Oralbase, including dexamethasone acetate, 0.02 g/mL.	No effect
CARBOXE ORABASE 20MG (CARBENOXOLONE) "SHITEH", including carbenoxolone, 0.01 g/mL.	No effect
STREPSILS LOZENGE, including 2,4-dichlorobenzyl alcohol	No effect

and amylmetacresol, 1 Tablet/5 mL.	
Watermelon frost, including herbal extract mixture, 0.02 g/mL.	No effect

* ref: Cancers 2020, 12, 2273; doi: 10.3390/cancers12082273.

- Clinical validation:

The clinical performance of EarlyFound® MMP-1 Rapid Strip Test was validated in 1029 subjects, including 326 OSCC patients and 703 OPMD patients.

Disease condition / Test result	OSCC	OPMD	Sub-total
Test Positive	260	97	357
Test Negative	66	606	672
Sub-total	326	703	1029 (Total)

Sensitivity: 79.8% (95% CI: 75.1%~83.8%)

Specificity: 86.2% (95% CI: 83.5%~88.6%)

PPV: 72.8% (95% CI: 67.9%~77.4%)

NPV: 90.2% (95% CI: 87.7%~92.3%)

Accuracy: 84.2% (95%CI: 81.8%~86.3%)

Symbols

IVD	In vitro diagnostic medical device	Σ	SUFFICIENT FOR<n>TESTS		DO NOT REUSE
	DO NOT USE IF PACKAGE IS DAMAGED		CAUTION		CONSULT INSTRUCTIONS FOR USE
REF	CATALOGUE NUMBER	LOT	BATCH CODE		USE BY DATE
	MANUFACTURER		KEEP AWAY FROM SUNLIGHT		KEEP DRY

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