

Analysis of the reliability of rapid diagnostic tests for varicella patients, including breakthrough cases

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Background

- The number of varicella cases has decreased by introducing a universal immunization program, but the number of breakthrough varicella (BV) cases has increased.
- DermaQuick® VZV is a rapid diagnostic test for varicella-zoster virus (VZV) infection developed by Japanese pharmaceutical company based on immunochromatography.
- Loop-mediated isothermal amplification (LAMP) is also useful for a rapid diagnostic test for varicella.

Aims of this study

- To clarify the reliability of direct VZV LAMP and DermaQuick® VZV as rapid diagnostic tests for varicella.
- To investigate the usefulness of saliva to diagnose for varicella.

Methods

1. Patients and samples collection

- Study period: April 2019 and January 2021.
- Subjects: Patients under 15 years old who were suspected varicella.

2. DermaQuick® VZV (Maruho Co., Ltd, Osaka, Japan)

- Skin swab samples which were soaked in reaction buffer was used. 3 drops were placed on the cartridge for 5-10 minutes at room temperature (Figure 1).

3. Direct LAMP assay

- Skin and oral swab samples were used **without DNA extraction**.
- All steps of this assay were completed **within 80 minutes**.

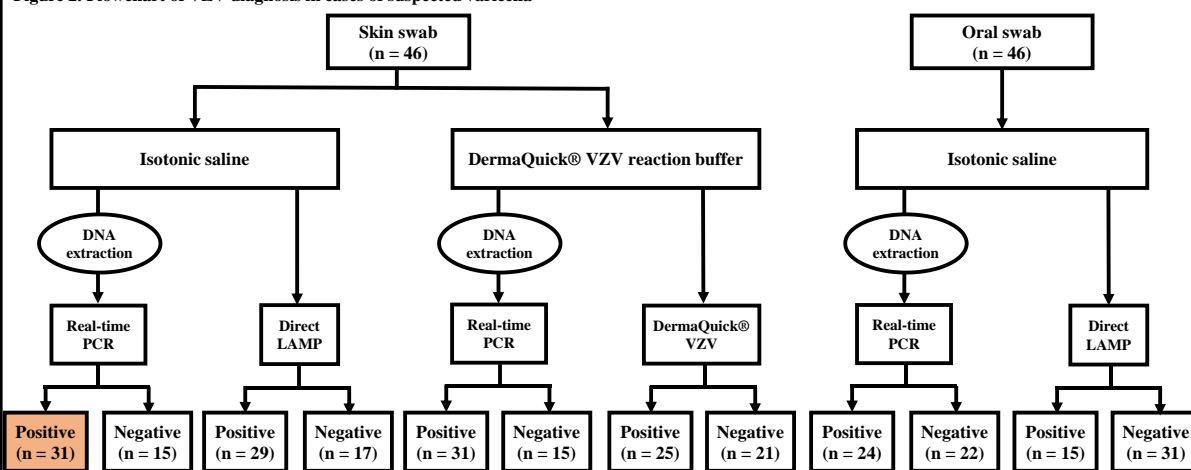
4. Real-time PCR and DNA extraction

- DNA was extracted from 200 µL of each samples using a QIAamp DNA blood mini kit (QIAGEN).
- The PCR reaction was performed using StepOne Real-Time PCR System (Thermo Fisher Scientific) in fast mode.

Figure 1. Diagnosis of VZV infection using DermaQuick® VZV for skin swab samples



Figure 2. Flowchart of VZV diagnosis in cases of suspected varicella



Results

- 31 patients (67.3%) were diagnosed with varicella** using real-time PCR of skin swabs (Figure 2. in orange box)
- 25 of 30 patients (83.3%)** received one dose of varicella vaccination, who were defined as **patients with BV**.

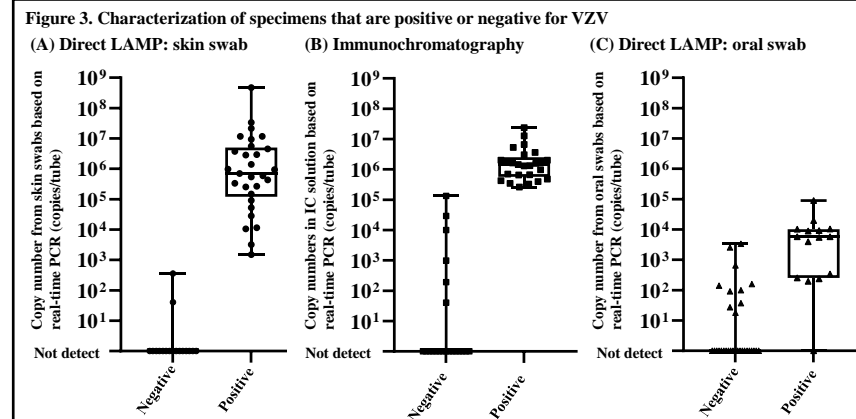


Table 1. Comparison of the performance of direct LAMP of skin swabs, immunochromatography of skin swabs, direct LAMP of oral swabs, and real-time PCR of oral swabs

		Real-time PCR skin swab		Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
		Positive	Negative				
Direct LAMP skin swab	Positive	29	0	93.6	100	100	88.2
	Negative	2	15	(79.3–98.9)	(79.6–100)	(88.3–100)	(65.7–97.9)
Immunochromatography skin swabs	Positive	25	0	80.7	100	100	71.4
	Negative	6	15	(63.7–90.8)	(79.6–100)	(86.7–100)	(50.0–86.2)
Direct LAMP oral swab	Positive	15	0	48.4	100	100	48.4
	Negative	16	15	(32.0–65.2)	(79.6–100)	(79.6–100)	(32.0–65.2)
Real-time PCR oral swab	Positive	23	1	74.2	93.3	95.8	63.6
	Negative	8	14	(56.6–86.3)	(70.2–99.7)	(79.8–99.8)	(43.0–80.2)

PPV: positive predictive value, NPV: negative predict value, CI: confidence interval.

Conclusion

- ✓ **The direct VZV LAMP method displays high sensitivity and specificity** and would be suitable for cheap and accurate point-of-care device for the rapid diagnosis of varicella.
- ✓ Although DermaQuick® VZV has the advantage to perform easily and quickly, it is not sensitive enough to diagnose BV precisely.
- ✓ Saliva samples are not suitable for diagnosis of BV, even if tested by real-time PCR. **More efficient saliva sampling methods and a DNA extraction step are needed** for direct VZV LAMP with saliva.

- The median copy number of VZV-test positive samples was 7.02×10^5 copies/tube for skin swabs (Figure 3A), 1.45×10^6 copies/tube for DermaQuick® VZV reaction buffer (Figure 3B), and 5.77×10^3 copies/tube for oral swabs (Figure 3C).
- The median copy numbers of VZV-test negative skin swabs for direct LAMP, immunochromatography, and oral swabs for direct LAMP were 1.97×10^2 copies/tube, 1.98×10^4 copies/tube, respectively.

- In 31 varicella patients, **29 (93.6%) were positive based on direct LAMP of skin swabs**.
- DermaQuick® VZV was positive in 25 (80.7%) of 31 varicella patients.
- Only 15 (48.4%) of the 31 varicella patients were positive with direct LAMP of oral swabs.
- The viral load of the false positive RT-PCR oral swab was 2.56×10^3 copies/tube (Table 1. in red circle).