

Effects of Reaction Time on Product Performance

1. Basic information:

Basic information			
Manufacturer		Beijing Hotgen Biotech Co.,Ltd.	
Experiment Site	Hotgen Biotech laboratory	Operator	5.1.2e
Date of Initiation and completion		2020.04.02	
Study protocol			
Samples		5 positive samples 5 negative samples	
Storage condition and Test interval		2~8°C	

2. Source and information of samples

2.1 Source of samples

Virus cultures: Academy of Military Medical Sciences

2.2 Preparation of samples

2.2.1 Collection of negative samples

Anterior nasal swabs of multiple healthy subjects shall be collected according to the sample collection method as specified in the IFU, diluted with the sample extraction buffer and then used as negative samples.

2.2.2 Preparation of positive samples

Dilute virus cultures with the sample extraction buffer 50, 100, 200, 400 and 800 times respectively to be used as positive samples 1~5.

3. Study protocol

Labeling process: Add 20 μ L of 2% potassium carbonate into 1mL of colloidal gold prepared from 0.04% chloroauric acid. Antibody concentration shall be labeled as 20 μ g/mL, and the labelling time is 10min. Block with 0.1% BSA for 5min.

Coating process: C-line: Goat anti Mouse IgG: 2.0 mg/mL; T-line: Antibody: 2.0 mg/mL. Coating buffer: 0.01M PB (pH7.2).

Observe test results respectively at 10min, 15min, 30min and 40min after sample injection, and study effects of reaction time on product performance.

4. Acceptance criteria

Testing results of negative and positive samples are obviously different, and positive samples with different concentrations have color gradients.

5. Testing results

Table 1 Testing results with different reaction time

Sample No.	Testing results with different reaction time				
	10min	13min	15min	30min	40min
Positive sample 1	++	+++	+++	+++	+++
Positive sample 2	++	++	++	++	++
Positive sample 3	+	++	++	++	++
Positive sample 4	+	+	+	+	+
Positive sample 5	±	+	+	+	-
Negative sample 1	-	-	-	-	-
Negative sample 2	-	-	-	-	-
Negative sample 3	-	-	-	-	-
Negative sample 4	-	-	-	-	-
Negative sample 5	-	-	-	-	-

6. Conclusions

There were no significant differences between low-value positive references and negative references at 10min reaction time, the coloration intensity of positive references with different concentrations was lower than those at 15min and 30min and the membrane surface showed red, indicating that the reaction was not complete at 10min; whereas, the coloration intensity had no changes at 15min and 30min, and positive references had obvious differences. After 40 minutes of reaction, there is no difference between the low-value positive control and negative control. Therefore, 15min has been selected as the reaction time of this sample test.