Studies on Calibration of Medical Carbon Dioxide Incubator

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Keywords: Medical Carbon Dioxide Incubator, Calibration, Temperature Deviation, Temperature Fluctuation, Temperature Uniformity, CO_2 Concentration Indication Error, CO_2 Concentration Control Error, Humidity.

Abstract: Medical carbon dioxide incubator is widely used in medical microbiological and biotechnological laboratories to simulate the growth environment for in vitro culture of cells or tissues of organisms. This article presents a novel design of calibration procedure to test the essential parameters of the medical carbon dioxide incubator such as "temperature deviation", "temperature fluctuation", "temperature uniformity", "CO2 concentration indication error", "CO2 concentration control error" and "humidity" in order to ensure the accuracy and reliability of the instrument. The research result can ensure the metrological traceability of medical carbon dioxide incubator by studying the instrument's key technical parameters and selecting the suitable metrological standards.

1 INTRODUCTION

Medical carbon dioxide incubator is widely used in medical microbiological and biotechnological laboratories to simulate the growth environment for in vitro culture of cells or tissues of organisms. Usually, the temperature inside the chamber is regulated at 37℃, and the CO_2 content of the atmosphere in the chamber is regulated at 5%. The main difference between medical carbon dioxide incubator and normal incubator is that the former can provide a certain concentration of CO_2 in order to meet the growth environment requirements of microorganism. Medical carbon dioxide incubator is essential metrological equipment for cell, tissue and bacterial culture in immunology, oncology, genetics and bioengineering researches. However, till now, national metrological verification regulation has not been issued applicable to the calibration of medical carbon dioxide incubator, therefore, and the corresponding traceability system of which has not been established yet.

The purpose of this paper is to study the influencing factors on essential technical parameter such as "temperature deviation", "temperature fluctuation", "temperature uniformity", "CO_2 concentration indication error", " CO_2 concentration control error" and "humidity" of medical carbon dioxide incubator, as well as to determine the measuring standards and to establish an applicable calibration procedure, in order to improve research conditions of medical laboratories, and furthermore, to establish the traceability system of medical carbon dioxide incubator.

2 CLIBRATION CONDITIONS

2.1 Environmental Conditions

Temperature: (15~35) ℃;

Humidity: ≤85%RH;

The environmental conditions shall also meet the requirements for the normal use of measuring standards and other supporting equipment.

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2.2 Measuring Standards and Other Supporting Equipment

Table 1: Measuring Standards and Other Supporting Equipment.

	Measuring Range	Technical Requirements
Standard Thermometer	(0~65) ℃	Definition: ≤0.1℃ MPE: ±0.2℃
Standard CO_2 Sensor	(0~20)%	Definition: ≤0.1% MPE: ±0.3%
Standard Humidity Sensor	(10~100)%RH	Definition: ≤0.1% MPE: ±2.0%RH

3 TEMPERATURE CLIBRATION

3.1 Temperature Calibration Procedure and Calculation Equations

Place 9 calibrated standard thermometers in the empty chamber of medical carbon dioxide with shelves in place as demonstrated in Figure 1. Locate standard thermometers (marked as 1, 2, 3, 4, 5, 6, 7, 8) in each of the eight corners of the incubator approximately 1/10 length of the side from each wall, and place the ninth standard thermometer (marked as 9) in the geometric center of the chamber.

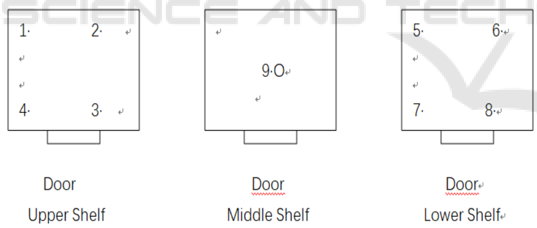

Figure1: Positioning of Standard Sensors

Set the control temperature at 37°C. Take 16 readings of each standard thermometer as well as the incubator's temperature indication value at regular intervals of 2min in 30 min during steady temperature condition. Calculate the temperature deviation, temperature fluctuation and uniformity according to equation (1), (2), (3):

$$\Delta t = \bar{t}_d - \bar{t}_0 \tag{1}$$

Δt —Temperature deviation, ℃;

$\bar{t}_d$ —Average indication temperature of medical carbon dioxide incubator, ℃;

$\bar{t}_0$ —The average temperature measured by standard thermometer 9 located in the geometric center, ℃.

$$\Delta t_f = \pm(t_{0\max} - t_{0\min})/2 \tag{2}$$

Δt_f —Temperature fluctuation, ℃;

$t_{0\max}$ —The maximum temperature value measured by standard thermometer 9 located in the geometric center, ℃;

$t_{0\min}$ —The minimum temperature value measured by standard thermometers located in the geometric center, ℃.

$$\Delta t_u = (t_{\max} - t_{\min})/2 \tag{3}$$

$t_{\max}$ —The maximum temperature reading taken by standard thermometers during 30min, ℃;

$t_{\text{i}\min}$ —The minimum temperature reading taken by standard thermometers during 30min, ℃;

Δt_u —Temperature uniformity, ℃.

3.2 Experimental Results of Temperature Calibration

Select several typical types of medical carbon dioxide incubators as the calibrated subjects. The experimental result is demonstrated in Table 2.

Table 2: Temperature Calibration Results (unit:℃).

Type	Deviation	Fluctuation	Uniformity
3111	0.2	±0.05	0.5
3131	0.2	±0.05	0.4
371	0.2	±0.1	0.5
HF240	-0.3	±0.1	0.4
HF240	0.3	±0.1	0.5
HF151	-0.2	±0.2	0.3
HF90	-0.3	±0.2	0.4
WJ-3-160	0.2	±0.05	0.3

The temperature deviation and fluctuation calibration results have met the metrological criterion set by YY 1621-2018 *Medical carbon dioxide incubator* of no more than ±0.3 ℃. The temperature uniformity calibration results have met the metrological criterion set by YY 1621-2018 *Medical carbon dioxide incubator* of no more than ±0.5 ℃.

4 CO_2 CONCENTRATION CALIBRATION

4.1 CO_2 Concentration Calibration Procedure and Calculation Equations

Place the standard CO_2 Senor (marked as O) in the geometric center of the incubator chamber as demonstrated in Figure 1.

Set the incubator's control temperature at 37°C and the CO_2 concentration value at 5%. Take 10 readings of the standard CO_2 sensor as well as the incubator's CO_2 concentration indication value at regular intervals of 15min 2 hours after the set value of temperature and CO_2 concentration has reached. Calculate the CO_2 concentration indication error and control error according to equation (4), (5):

$$C_d = \overline{C_d} - C_s \quad (4)$$

C_d —CO_2 concentration indication error, %;

$\overline{C_d}$ —Average value of 10 CO_2 concentration indication readings of the incubator, %;

C_s —CO_2 concentration set value of incubator, %.

$$C_c = \overline{C} - C_s \quad (5)$$

C_c —CO_2 concentration control error, %;

$\overline{C}$ —Average value of 10 readings of CO_2 sensor, %.

4.2 Experimental Results of CO_2 Concentration Calibration

Select several typical types of medical carbon dioxide incubators as the calibrated subjects. The experimental result is demonstrated in Table 3:

Table 3: CO_2 Concentration Calibration Results (unit: %).

Type	Indication Error	Control Error
3111	0.1	0.3
3131	-0.2	-0.4
371	0.2	0.4
HF240	0.1	0.2
HF240	0.1	0.1
HF151	0.2	-0.4
HF90	-0.1	-0.2
WJ-3-160	0.1	0.1

The CO_2 concentration indication error calibration results have met the metrological criterion set by YY 1621-2018 Medical *carbon dioxide incubator* of no more than ±0.2%, and the CO_2 concentration control error calibration results have met the metrological criterion set by YY 1621-2018 *Medical carbon dioxide incubator* of no more than ±0.5%.

5 HUMIDITY CALIBRATION

5.1 Humidity Calibration Procedure

Place the standard humidity Senor (marked as O) in the geometric center of the incubator chamber as demonstrated in Figure 1.

Set the incubator's control temperature at 37°C and the CO_2 concentration value at 0%. Take the reading of the standard humidity sensor 2 hours after the set value of temperature.

5.2 Experimental Results of Humidity Calibration

Select several typical types of medical carbon dioxide incubators as the calibrated subjects. The experimental result is demonstrated in Table 4.

Table 4: Humidity Calibration Results (unit: %).

Type	Humidity measured by Standard Sensor
3111	97.6
3131	98.1
371	99.0
HF240	97.9
HF240	98.2
HF151	99.1
HF90	99.0
WJ-3-160	99.5

The humidity calibration results have met the metrological criterion set by enterprise standards of no less than 95%.

6 UNCERTAINTY ANALYSIS

6.1 Uncertainty Analysis on Temperature Measurement

6.1.1 Standard Uncertainty Introduced by Temperature Measurement Repeatability

Select a type 3111 medical carbon dioxide incubator to conduct the temperature calibration procedure presented in Chapter 3 at the set value of 37℃. The obtained values are demonstrated in Table 5:

Table 5: Temperature Repeatability Test Result (unit: ℃).

No.	Measured Temperature
1	36.8
2	36.9
3	36.8
4	36.7
5	36.8
6	36.7
7	36.9
8	36.8

9	36.8
10	36.7
Sum	367.9
$\overline{t}$	36.79

Calculates the standard deviation with Bessel formula:

$$s(t)=\sqrt{\frac{\sum_{i=1}^{n}(t_i-\bar{t})^2}{n-1}}=\sqrt{\frac{\sum_{i=1}^{10}(t_i-\bar{t})^2}{10-1}}=0.074℃ \tag{6}$$

16 readings shall be taken when the medical carbon dioxide incubator is calibrated in practical use. So the standard uncertainty introduced by measurement repeatability is as follows:

$$u_1=\frac{s_{(t)}}{\sqrt{16}}=0.0185℃ \tag{7}$$

6.1.2 Standard Uncertainty Introduced by the Resolution of Standard Thermometer

The resolution of standard thermometer is 0.1°C, considering uniform distribution, the standard uncertainty introduced by the resolution is as follows:

$$u_2=\frac{0.1}{2\sqrt{3}}=0.029℃ \tag{8}$$

6.1.3 Standard Uncertainty Introduced by the Maximum Permissive Error of Standard Thermometer

The maximum permissive error of standard thermometer is ±0.2°C, considering uniform distribution, the standard uncertainty introduced by maximum permissive error is as follows:

$$u_3=\frac{0.2}{2\sqrt{3}}=0.05℃ \tag{9}$$

6.1.4 Synthetic Uncertainty of Temperature Measurement

The uncertainty components analyzed are shown in Table 6:

Table 6: Summary of Uncertainty Components of Temperature Measurement (Unit: °C).

Source of Standard Uncertainty	Uncertainty Type	Uncertainty
Measurement Repeatability	A	0.0185
Resolution of Standard Thermometer	B	0.029
The Maximum Permissive Error of Standard Thermometer	B	0.058

Since the standard uncertainty introduced by the resolution of standard thermometer $u_2=0.029℃$ is positively correlated with the uncertainty introduced by measurement repeatability $u_1=0.0185℃$, therefore, only the standard uncertainty introduced by resolution of standard thermometer u_2 is considered, when synthetic uncertainty is calculated as follows:

$$u_c=\sqrt{u_2+u_3}=0.064℃ \tag{10}$$

6.1.5 Extended Uncertainty of Temperature Measurement

Take the inclusion factor k=2 (confidence probability is 95%), the extended uncertainty is calculated as follows:

$$U=ku_c=0.064\times 2=0.128\approx 0.13℃ \tag{11}$$

6.2 Uncertainty Analysis on CO_2 Concentration Measurement

6.2.1 Standard Uncertainty Introduced by CO_2 Concentration Measurement Repeatability

Select a type 3111 medical carbon dioxide incubator to conduct the CO_2 concentration calibration procedure presented in Chapter 4 at the set value of 5%. The obtained values are demonstrated in Table 7:

Table 7: CO_2 Concentration Repeatability Test Result.

No.	Measured CO_2 Concentration/%
1	4.8
2	4.7
3	4.8
4	4.7
5	4.9
6	4.6
7	4.7
8	4.8
9	4.8
10	4.7
Sum	47.5
$\overline{t}$	4.75

Calculates the standard deviation with Bessel formula:

$$s(t)=\sqrt{\frac{\sum_{i-1}^{n}(t_i-\bar{t})^2}{n-1}}=\sqrt{\frac{\sum_{i=1}^{10}(t_i-\bar{t})^2}{10-1}}=0.085\% \quad (12)$$

10 readings shall be taken when the medical carbon dioxide incubator is calibrated in practical use. So the standard uncertainty introduced by measurement repeatability is as follows:

$$u_1=\frac{s_{(t)}}{\sqrt{10}}=0.027\% \quad (13)$$

6.2.2 Standard Uncertainty Introduced by the Resolution of Standard CO_2 Sensor

The resolution of standard CO_2 sensor is 0.1%, considering uniform distribution, the standard uncertainty introduced by the resolution is as follows:

$$u_2=\frac{0.1}{2\sqrt{3}}=0.029\% \quad (14)$$

6.2.3 Standard Uncertainty Introduced by the Maximum Permissive Error of Standard CO_2 Sensor

The maximum permissive error of standard CO_2 sensor is ±0.3%, considering uniform distribution, the standard uncertainty introduced by maximum permissive error is as follows:

$$u_3=\frac{0.3}{2\sqrt{3}}=0.087\% \quad (15)$$

6.2.4 Synthetic Uncertainty of CO_2 Concentration Measurement

The uncertainty components analyzed are shown in Table 8:

Table 8: Summary of Uncertainty Components of CO_2 Concentration Measurement.

Source of Standard Uncertainty	Uncertainty Type	Uncertainty (%)
Measurement Repeatability	A	0.31
Resolution of Humidity Sensor	B	0.029
The Maximum Permissive Error of Humidity Sensor	B	0.58

Since the standard uncertainty introduced by the resolution of standard CO_2 sensor $u_2=0.029\%$ is positively correlated with the uncertainty introduced by measurement repeatability $u_1=0.027\%$, therefore, only the standard uncertainty introduced by resolution of standard CO_2 sensor u_2 is considered, when synthetic uncertainty is calculated as follows:

$$u_c=\sqrt{u_2+u_3}=0.092\% \quad (16)$$

6.2.5 Extended Uncertainty of CO_2 Concentration Measurement

Take the inclusion factor k=2 (confidence probability is 95%), the extended uncertainty is calculated as follows:

$$U=ku_c=0.092\times2=0.184\approx0.2℃ \quad (17)$$

6.3 Uncertainty Analysis on Humidity Measurement

6.3.1 Standard Uncertainty Introduced by Humidity Measurement Repeatability

Select a type 3111 medical carbon dioxide incubator to conduct the humidity calibration procedure presented in Chapter 5. The obtained values are demonstrated in Table 9:

Table 9: Humidity Repeatability Test Result.

No.	Humidity measured by Standard Sensor /%
1	97.8
2	97.9
3	98.0
4	98.2
5	97.6
6	97.9
7	97.7
8	98.5
9	97.5
10	97.6
Sum	978.7
$\bar{t}$	97.87

Calculates the standard deviation with Bessel formula:

$$s(t)=\sqrt{\frac{\sum_{i-1}^{n}(t_i-\bar{t})^2}{n-1}}=\sqrt{\frac{\sum_{i=1}^{10}(t_i-\bar{t})^2}{10-1}}=0.31\% \quad (18)$$

6.3.2 Standard Uncertainty Introduced by the Resolution of Standard Humidity Sensor

The resolution of standard humidity sensor is 0.1%, considering uniform distribution, the standard uncertainty introduced by the resolution is as follows:

$$u_2 = \frac{0.1}{2\sqrt{3}} = 0.029\% \tag{19}$$

6.3.3 Standard Uncertainty Introduced by the Maximum Permissive Error of Standard Humidity Sensor

The maximum permissive error of standard humidity sensor is ±2.0%, considering uniform distribution, the standard uncertainty introduced by maximum permissive error is as follows:

$$u_3 = \frac{2}{2\sqrt{3}} = 0.58\% \tag{20}$$

6.3.4 Synthetic Uncertainty of Humidity Measurement

The uncertainty components analyzed are shown in Table 10:

Table 10: Summary of Uncertainty Components of CO_2 Concentration Measurement.

Source of Standard Uncertainty	Uncertainty Type	Uncertainty (%)
Measurement Repeatability	A	0.027
Resolution of Standard CO_2 Sensor	B	0.029
The Maximum Permissive Error of CO_2 Sensor	B	0.087

Since the standard uncertainty introduced by the resolution of standard humidity sensor $u_2 = 0.029\%$ is positively correlated with the uncertainty introduced by measurement repeatability $u_1 = 0.31\%$, therefore, only the standard uncertainty introduced by measurement repeatability u_1 is considered, when synthetic uncertainty is calculated as follows:

$$u_c = \sqrt{u_1 + u_3} = 0.66\% \tag{21}$$

6.3.5 Extended Uncertainty of Humidity Measurement

Take the inclusion factor *k*=2 (confidence probability is 95%), the extended uncertainty is calculated as follows:

$$U = ku_c = 0.66 \times 2 = 1.32 \approx 1.3\% \tag{22}$$

7 CONCLUSION

The article studies the key technical parameters such as "temperature deviation", "temperature fluctuation", "temperature uniformity", "CO_2 concentration indication error", " CO_2 concentration control error" of medical carbon dioxide incubator, determines the appropriate standards and supporting instruments for measuring, and performs the novel calibration procedure presented in this article on several typical types of medical carbon dioxide incubators widely used in China. The testing results meet the requirements of national standard YY1621-2018.

Therefore, the article presents a feasible procedure for the periodic calibration of medical carbon dioxide incubator in order to establish the metrological traceability system of the instrument. Further work is worth to be done to improve the calibration method of medical carbon dioxide incubator.

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