Evaluation of Uncertainty Type A of a temperature scanner used for Pyrogen Test.

Ing. Miguel Alberto López Regalado[1], Tec. Orlando Pérez Jomolca[2]

[1]National Center of Biologycal Products (BioCen), Cuba. Chief of Metrology Laboratory.
Tel. 53-47682201. E-mail miguel.lopez@biocen.cu. [2]Mycron Metrology Laboratories, Havana, Cuba.
Specialist in Temperature Calibrations.

Abstract

Pyrogen test is a mandatory regulation for pharmaceutical manufacturers in some countries. It must be carried out according to an approved Pharmacopeia. The temperature margins for this test are very tigh (0.5 °C). To be sure that the temperature measurements meet the required metrological specifications, the scanner must be calibrated periodically and its uncertainty estimated. Being this test differential, the exact value of temperature is not so important as the difference between temperature at the beginning and the end of the test, so it's of capital importance that the stability and repeatability of the measurements be determined. In this work, the uncertainty of a ELLAB PyroMon UI128 temperature monitor is evaluated by type A evaluation method. This monitor can scan up to 80 thermocouples to measure the rectal temperature of rabbits during the pyrogen test. The basic method used was the comparison between the scanner's readings and a standard platinum resistance thermometer (SPRT), in a well stabilized stirred bath. All measurements are traceable to international standards. Two independent tests were done with all the probes; firstly, 15 measurement were done, in 5 min steps, to determine the stability in a period of time of the scanner accuracy. Then 10 measurements were done alternating the probes from 38.5 °C to 0 °C (ice bath) and viceversa, to determine the instrument repeatability. Both tests results were analyzed statistically, and the uncertainty was determined according to GUM 2008. It was found that the obtained uncertainty matches the requirements for the Pyrogen Test , achieving a maximum value of 0.025 °C once combined with that of the standard instrument. This result allow us to accept the instrument as valid for the realization of this test.

Keywords: pyrogen test, temperature, calibration, uncertainty

Introduction

Pyrogen test is a mandatory regulation for pharmaceutical manufacturers in some countries. It must be carried out according to an approved Pharmacopeia, which is agreed with the customer [1-3]. It is very important and mandatory that any parenteral pharmaceutical preparation (PFP) be pyrogen-free, so, in order to demonstrate that any PFP is pyrogen-free the pyrogen test is done. The pyrogen test consist in monitoring the temperature of a test animal (rabbits in this case) by inserting a probe in the animal rectum, before and after the PFP was injected. If the rectal temperature of the animal remains within a temperature interval of 0.5 °C for a certain period of time after the injection of the PFP to the rabbit, then the PFP is pyrogen-free, however, if the temperature increases by more than 0.5 °C then the PFP could not be safe and the test should be repeated. If in the repetition the result is the same, one must discard the PFP batch, due to pyrogen contamination.

It is common practice to use a pyrogen test processor which is a scanning device which scan the temperature of all of the thermocouples used as probes to measure the rectal temperature of the animals. To be sure that the temperature measurements meet the required metrological specifications, the scanner must be calibrated periodically and its uncertainty estimated. Being this test differential, the error itself is not so important cause it will be the same both at the beginning and end of the test, but it's of capital importance that the stability and repeatability of the measurements be determined with as low uncertainty as possible. The aim of this work is to determine the contributions of uncertainty due to stability and repeatability that should be taken into account during the calibration of this scanning equipment, and to demonstrate that they are smaller than 10 % of the pyrogen test acceptance criterion of 0.5 °C.

Materials and methods

The device under test was a ELLAB A/S PyroMon UI128 Pyrogen Test Processor, from Denmark. This monitor can scan up to 80 thermocouples, but in our case it has 64 thermocouples only. As standard it was used a HART-Scientific standard platinum resistance thermometer (SPRT), type 5416, and a FLUKE 1502A Tweener. All measurements are traceable to Mendeleyev Institute for Metrology (VNIIM).

The controlled temperature medium at 38.5 °C was an ERTCO TCS 35-200 controlled stirred bath, with temperature stability of 0.007 °C. This bath was filled with distilled water as thermometric fluid. The stabilized temperature medium at 0 °C was an ice bath prepared with distilled water in two phases, both solid and liquid, in thermal equilibrium [5]. Room temperature and relative humidity of the air were measured during the test, using a Control Company digital thermo-hygrometer. Temperature remained within the range from 20 to 23 °C and relative humidity remained within the range from 55 to 65 %.

Two independent tests were performed: stability and repeatability. In the stability test, all of the thermocouples and the standard probe were immersed in the controlled temperature medium [6] at 38.5 °C. Once stabilized, 15 measurements were done, in 5 min steps, for each thermocouple, to determine the stability in a period of time of the scanner accuracy.

In the repeatability test, the capacity to measure temperature correctly after a temperature change was challenged. All of the thermocouples and the standard probe were immersed in the controlled temperature medium [6] at 38.5 °C. Once stabilized, the temperature of each thermocouple was read, and then all of the thermocouples and the standard probe were immersed in the ice bath. Once the readings stabilized, the thermocouples and the standard probe were immersed again in the medium at 38.5 °C. Again the temperature was read after stabilization. This procedure was repeated 10 times.
All of the repetitions for each thermocouple, in both tests were analyzed statistically to determined their Type A uncertainty contribution. The average or arithmetic mean, the error and standard uncertainty were evaluated.

The average was evaluated by the formula [4], [7]:

$$\bar{q} = \frac{1}{n}\sum_{k=1}^{n} q_k \qquad (1)$$

Where:
$\bar{q}$: average or arithmetic mean
q_k :each independent measurement.
n : number of measurements

The error was calculated by the formula:

$$e = \bar{q} - T_s \qquad (2)$$

Where:
e : error of the thermocouple
$\bar{q}$:average or arithmetic mean.
Ts :temperature measured by the standard.

The standard uncertainty of each thermocouple was evaluated by the estimation of the experimental variance of the measurements, which estimates the variance σ^2 of the probability distribution of *q*, and is given by the formula [4], [7]:

$$s^2(q_k) = \frac{1}{n-1}\sum_{j=1}^{n}\left(q_j - \bar{q}\right)^2 \qquad (3)$$

Where:
$s^2(q_k)$: experimental variance of the measurements
$\bar{q}$:average or arithmetic mean.
q_j: each independent measurement
n : number of measurements

And then, the best estimate of the variance of the mean [4], is given by:

$$s^2(\overline{q}) = \frac{s^2(q_k)}{n} \tag{4}$$

Where:
$s^2(q_k)$: experimental variance of the measurements.
$s^2(\overline{q})$: variance of the mean.
n : number of measurements.

The experimental standard deviation of the mean of the series of observations is then evaluated as the positive square root of the variance of the mean by the formula [4], [7]:

$$s(\overline{q}) = \sqrt{\frac{s^2(q_k)}{n}} \tag{5}$$

This result is the Type A standard uncertainty for each thermocouple.

Both standard uncertainties (stability and repeatability) are then combined with the uncertainty reported in the Calibration Certificate of the standard instruments, in order to evaluate the total contribution to the measurement of each thermocouple. The combined standard uncertainty is evaluated by the following formula [4], [6], [7] :

$$u_c = \sqrt{(u_c\,(stab))^2 + (u_c\,(rep))^2 + (u_c(stan))^2} \tag{6}$$

Where:
u_c : combined standard uncertainty of the measurements of each thermocouple.
u_c (stab): standard uncertainty of the measurements of each thermocouple due to stability.
u_c(rep): standard uncertainty of the measurements of each thermocouple due to repeatibility.
u_c(stan): standard uncertainty reported in the calibration certificate of the standard instrument.

***Note:** The uncertainty contribution of the stability of the bath is not taken into account because it is of the same order than that of the measurements so it can be considered that the differences in the readings are due to the bath stability* [5].

Results and Discussion

The data about stability and repeatability of results that were evaluated for each thermocouple is shown on Table 1 and Table 2. These results showed that all of the thermocouples measurements are affected by a standard uncertainty lower than 10 mK due to both stability in time and repeatability during temperature changes.

Table 1. Stability test results for all of the thermocouples (T/C)

T/C No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Average	38.482	38.513	38.477	38.524	38.507	38.519	38.519	38.517
Error	-0.018	0.013	-0.023	0.024	0.007	0.019	0.019	0.017
u_c	0.00490	0.00658	0.00463	0.00773	0.00630	0.00631	0.00496	0.00454
T/C No.	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Average	38.475	38.489	38.537	38.573	38.485	38.487	38.487	38.497
Error	-0.025	-0.011	0.037	0.073	-0.015	-0.013	-0.013	-0.003
u_c	0.00631	0.00792	0.00891	0.00843	0.00515	0.00897	0.00760	0.00735
T/C No.	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Average	38.559	38.541	38.517	38.503	38.542	38.503	38.506	38.518
Error	0.059	0.041	0.017	0.003	0.042	0.003	0.006	0.018
u_c	0.00621	0.00556	0.00746	0.00746	0.00678	0.00566	0.00584	0.00312
T/C No.	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
Average	38.523	38.519	38.509	38.523	38.521	38.521	38.495	38.543
Error	0.023	0.019	0.009	0.023	0.021	0.021	-0.005	0.043
u_c	0.00492	0.00401	0.00431	0.00316	0.00350	0.00589	0.00363	0.00492
T/C No.	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
Average	38.485	38.511	38.516	38.504	38.519	38.525	38.531	38.521
Error	-0.015	0.011	0.016	0.004	0.019	0.025	0.031	0.021
u_c	0.00639	0.00267	0.00434	0.00466	0.00284	0.00524	0.00597	0.00714
T/C No.	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
Average	38.539	38.490	38.524	38.517	38.503	38.519	38.497	38.524
Error	0.039	-0.010	0.024	0.017	0.003	0.019	-0.003	0.024
u_c	0.00551	0.00816	0.00668	0.00589	0.00530	0.00836	0.00492	0.00660
T/C No.	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**
Average	38.496	38.491	38.523	38.503	38.533	38.510	38.525	38.503
Error	-0.004	-0.009	0.023	0.003	0.033	0.010	0.025	0.003
u_c	0.00576	0.00463	0.00549	0.00797	0.00615	0.00683	0.00910	0.00651
T/C No.	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**
Average	38.517	38.508	38.509	38.482	38.519	38.495	38.541	38.507
Error	0.017	0.008	0.009	-0.018	0.019	-0.005	0.041	0.007
u_c	0.00589	0.00829	0.00613	0.00500	0.00565	0.00446	0.00463	0.00463

Table 2. Repeatability test results for all of the thermocouples (T/C)

T/C No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Average	38.490	38.490	38.503	38.555	38.492	38.489	38.464	38.482
Error	-0.015	-0.015	-0.002	0.051	-0.012	-0.016	-0.040	-0.022
u_c	0.0088	0.0075	0.0075	0.0060	0.0081	0.0078	0.0087	0.0049
T/C No.	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Average	38.564	38.538	38.506	38.489	38.497	38.540	38.505	38.501
Error	0.059	0.034	0.0015	-0.0155	-0.0075	0.0355	0.0005	-0.0035
u_c	0.0085	0.0047	0.0087	0.0078	0.0080	0.0082	0.0065	0.0069
T/C No.	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Average	38.526	38.537	38.503	38.501	38.519	38.523	38.505	38.512
Error	0.0215	0.0325	-0.0015	-0.0035	0.014	0.019	0.000	0.007
u_c	0.0070	0.0068	0.0047	0.0038	0.0072	0.0065	0.0050	0.0033
T/C No.	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
Average	38.480	38.531	38.471	38.520	38.523	38.502	38.527	38.519
Error	-0.024	0.026	-0.033	0.015	0.019	-0.003	0.023	0.014
u_c	0.0058	0.0067	0.0066	0.0058	0.0065	0.0079	0.0079	0.0082
T/C No.	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
Average	38.539	38.554	38.543	38.532	38.512	38.564	38.524	38.531
Error	0.035	0.049	0.038	0.028	0.008	0.060	0.020	0.026
u_c	0.0053	0.0064	0.0080	0.0079	0.0074	0.0087	0.0085	0.0060
T/C No.	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
Average	38.542	38.515	38.540	38.523	38.530	38.523	38.546	38.539
Error	0.038	0.011	0.035	0.019	0.025	0.019	0.042	0.035
u_c	0.0065	0.0076	0.0033	0.0072	0.0075	0.0072	0.0050	0.0053
T/C No.	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**
Average	38.476	38.533	38.519	38.538	38.515	38.540	38.504	38.513
Error	-0.029	0.029	0.015	0.033	0.011	0.036	0.000	0.008
u_c	0.0076	0.0052	0.0085	0.0077	0.0069	0.0075	0.0067	0.0060
T/C No.	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**
Average	38.517	38.513	38.520	38.474	38.525	38.480	38.526	38.503
Error	0.012	0.008	0.016	-0.030	0.020	-0.024	0.021	-0.002
u_c	0.0081	0.0090	0.0075	0.0048	0.0049	0.0047	0.0082	0.0089

Once combined with the uncertainty of the standard according to equation (6), we found that all of the thermocouples met the proposed acceptance criterion for the standard combined uncertainty, as for all of them this parameter is lower than 0.025 °C. This data is shown on Table 3.

Table 3. Combined standard uncertainty for all of the thermocouples (T/C)

T/C	u_c(°C)	T/C	u_c(°C)	T/C	u_c(°C)	T/C	u_c(°C)
1	0.0105	17	0.0098	33	0.0088	49	0.0100
2	0.0104	18	0.0092	34	0.0075	50	0.0075
3	0.0093	19	0.0093	35	0.0095	51	0.0105
4	0.0102	20	0.0088	36	0.0096	52	0.0114
5	0.0106	21	0.0103	37	0.0084	53	0.0097
6	0.0104	22	0.0091	38	0.0105	54	0.0105
7	0.0104	23	0.0082	39	0.0108	55	0.0116
8	0.0073	24	0.0054	40	0.0097	56	0.0093
9	0.0110	25	0.0081	41	0.0090	57	0.0104
10	0.0096	26	0.0083	42	0.0115	58	0.0126
11	0.0130	27	0.0084	43	0.0080	59	0.0101
12	0.0119	28	0.0072	44	0.0097	60	0.0075
13	0.0099	29	0.0079	45	0.0096	61	0.0080
14	0.0125	30	0.0103	46	0.0114	62	0.0071
15	0.0104	31	0.0091	47	0.0076	63	0.0099
16	0.0105	32	0.0100	48	0.0089	64	0.0104

Conclusions

The uncertainty contribution of the PyroMon UI128 Pyrogen Test Processor of our lab is acceptable as it is smaller than 25 mK for all of the thermocouples.

This procedure can be applied to any other temperature monitor in order to evaluate its uncertainty contribution during the measurement process.

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