Combined use of non-laboratory methods for the practical diagnosis of endometritis in postpartum dairy cows

Hiromi Kusaka, Taketoki Kimura, Namiko Nishimoto, Minoru Sakaguchi

1Laboratory of Theriogenology, School of Veterinary Medicine, Kitasato University, Towada, Aomori, Japan
2Livestock Research Institute of the Aomori Prefectural Industrial Technology Research Center, Noheji, Aomori, Japan

Correspondence
Hiromi Kusaka, Laboratory of Theriogenology, School of Veterinary Medicine, Kitasato University, Towada, Aomori, Japan.
E-mail: kusaka@vmas.kitasato-u.ac.jp

Abstract

Background: Postpartum endometritis can cause subfertility in cows and reduce the reproductive performance of dairy herds. Thus, there is a critical need to improve the accuracy of the diagnosis of this disease, particularly in dairy cows.

Objectives: This study confirmed the effects of the combined use of two non-laboratory methods: Metricheck device (MT) and ultrasonography (UT) to detect signs of endometritis and therefore predict the likelihood of pregnancy.

Methods: The reproductive tract of 67 lactating Holstein cows (25 primiparous and 42 multiparous) was observed at 4 and 6 weeks postpartum using the MT and UT. The cows with flecks and more purulent material in the vaginal discharge were defined as MT-positive, and the cows with uterine echogenic contents were defined as UT-positive. The combined definition was also used; both the UT and MT results or either the UT or MT result had to be positive for the final decision to be positive. The true-negative cows were determined as both MT-UT-negative.

Results and discussion: When comparing the diagnostic results obtained by MT and UT, 37.3% and 32.8% of the total cows showed conflicting results at 4 and 6 weeks postpartum. The proportion of non-pregnant cows until 200 days postpartum was significantly greater in both MT-UT-positive cows than in the true negative cows. The hazard ratios of pregnancy rate were 0.37 for both MT-UT-positives relative to the true negatives at 4 weeks postpartum. Similar significant differences were not detected using the MT or UT alone. These results indicate that a more severe effect on the chance of pregnancy could be detected in cows that are both MT-UT-positive compared with those positive for MT or UT alone.

Conclusions: The combined use of MT and UT could support veterinary practitioners in diagnosing endometritis, potentially improving the accuracy of predicting subsequent reproductive performance.

KEYWORDS
endometritis, dairy cows, diagnostic methods
Uterine diseases are commonly observed in dairy cows postpartum, with over 90% of them hosting bacteria in their uterine lumen within the first two weeks after calving (Sheldon et al., 2002). The majority of cows eliminate these bacteria spontaneously; however, some are unable to clear this infection naturally, subsequently developing postpartum endometritis, which can lead to subfertility in cows. The number of services per conception increased from 2.1 to 2.4 and the interval from calving to conception also extended by 31 days on average for the cows with endometritis compared with the cows without (Noakes et al., 2019). There is an important need for veterinary practitioners to be able to diagnose endometritis accurately and to provide appropriate treatment, which could improve the overall reproductive performance of dairy herds; however, a practical gold standard for the diagnosis of endometritis is still absent.

Clinical endometritis is defined as the presence of a purulent uterine discharge detectable in the vagina of cattle 21 days postpartum or a mucopurulent discharge detectable in the vagina after 26 days postpartum (Sheldon et al., 2006). Recently, many authors have moved away from using the term clinical endometritis, preferring the term purulent vaginal discharge (PVD) because it is more descriptive and covers a wider range of conditions, including endometritis, vaginitis and cervicitis (Dubuc et al., 2010).

In clinical practice, diagnostic methods need to be low-cost, non-laboratory, non-skilled and on-farm convenience tests. Metricheck devices (MT) have been developed as a practical tool for detecting PVD, known to have a negative influence on fertility in cows (McDougall et al., 2011; Williams et al., 2005), acting as an alternative to traditional vaginoscopy (McDougall et al., 2007). MT is easy to insert and clean and is as efficient as vaginoscopy for detecting vaginal materials. However, the use of an MT in isolation fails to detect cows that have no vaginal discharge but abnormal intrauterine fluid (Šavc et al., 2016).

With the widespread use of ultrasonography (UT) in dairy reproduction, it has been applied to monitor the genital status not only in the ovaries but also in the uterus. UT was developed and applied in field conditions to distinguish abnormal uterine status from normal status, directly confirming the presence or absence of echogenic fluid in the uterus. The intrauterine echogenic fluid may be associated with bacterial growth and delayed clearance of inflammatory response substances (Mateus et al., 2002a; Mateus et al., 2002b) and could be a promising indicator for subsequent reproductive performance (Barlund et al., 2008; López-Helguera et al., 2012; Kasimanickam et al., 2004).

Recently, the cytobrush technique (CYT) has been developed as a more accurate and objective method for evaluating uterine endometrial health status and predicting future subfertility. For example, reductions in first service conception rates and final pregnancy rates have been reported in cows diagnosed with cytological endometritis using the CYT (Kasimanickam et al., 2004; McDougall et al., 2011; Senosy et al., 2012). However, this technique is a laboratory method that requires skilful sampling by veterinarians. Therefore, some previous studies have compared the availability of the CYT and one of the other methods (MT or UT) to diagnose postpartum endometritis (Dubuc et al., 2010; Kasimanickam et al., 2004; McDougall et al., 2007, 2011; Meira et al., 2012).

In a previous study, we compared the diagnosis of endometritis obtained using three different diagnostic methods: CYT, MT and UT (Kusaka et al., 2020). As a result, we found little agreement among these methods in terms of diagnosing endometritis between 2 and 6 weeks after calving. Furthermore, the results implied that the combined use of MT and UT could provide primarily equivalent results as CYT; the highest sensitivity was achieved when positive individuals were identified as either MT-positive or UT-positive, whereas the highest specificity occurred when results were positive by both MT and UT methods. Thus, the former application is recommended when attempting to decrease false-negative results, and the latter is recommended when attempting to decrease false-positive results (Kusaka et al., 2020). However, the usefulness of the combined use of MT and UT has yet to be verified in many animals, considering the subsequent reproductive performance.

Thus, this study focused on the two non-laboratory methods, MT and UT, that can be used in field conditions. We aimed to confirm the efficiency of single or combined use of the two non-laboratory methods to detect signs of endometritis and therefore predict the likelihood of pregnancy in dairy cows.

## 2 MATERIALS AND METHODS

### 2.1 Cows and herd management

Data presented in this study were collected from 79 (28 primiparous and 51 multiparous) lactating Holstein cows. The cows calved between February 2016 and March 2020 at the Livestock Research Institute of the Aomori Prefectural Industrial Technology Research Centre (Noheji, Aomori, Japan). Cows were housed in a free-stall barn after parturition, fed a diet formulated according to the Japanese Feeding Standard for Dairy Cattle (National Agriculture and Food Research Organization, 2006), and milked twice a day (at 08:30 and 17:00). The rolling herd average of milk yield was 10,320 kg per cow. Data from 12 cows were eliminated from the total 79 cows; 1, 1 and 2 cows experienced stillbirth, retained placenta and respiratory disease, respectively, and the values for the uterine examination of eight cows were missing. Consequently, 67 cows were enrolled in the present analyses, containing one cow with twin births.

### 2.2 Experimental design

The reproductive tracts of all cows were observed at 4 and 6 weeks postpartum by one veterinarian (ranging from 26 to 30 and 40 to 44 days postpartum, respectively). First, vaginal discharge was collected by MT, followed by UT on each experimental day. Vaginal discharge was
collected using an MT device (Simcro Tech, Hamilton, New Zealand), as previously described (McDougall et al., 2007), with some modifications. Then, the devices were rinsed in an antiseptic solution between cows. Intravenous fluid was monitored using a real-time linear array ultrasound scanner equipped with a 7.5 MHz rectal probe (HS-1600; Honda Electronics, Tokyo, Japan). Scanning was performed carefully and slowly along the dorsal/lateral surface of the uterus, and the equipment was supplied with image freeze to observe intravenous fluid at the base of each horn (approximately 5 cm anterior to the uterine body).

The vaginal material within the concave surface of the device and/or adherent to the convex surface obtained by MT was assessed for categories on a scale from 0 to 5 (0 = no discharge, 1 = clear mucus, 2 = flecks of purulent material, 3 = mucopurulent but <50% purulent material, 4 = mucopurulent with >50% purulent material, and 5 = mucopurulent with an odour). The intravenous fluid observed by UT was categorised on a scale of 0 to 2 (0 = anechoic, 1 = echogenic compact contents, 2 = echogenic fluctuant contents) (López-Helguera et al., 2012).

### 2.3 Diagnostic criteria

In this study, four diagnostic criteria were defined. First, cows with MT categories ≥2 were determined to be MT-positive, while the others were considered MT-negative (McDougall et al., 2007). Second, cows with UT categories ≥1 were determined as UT-positive, while the others were considered UT-negative (López-Helguera et al., 2012).

In addition, the combined definition of two non-laboratory methods, MT and UT, was used. Namely, both the UT and MT results or either the UT or MT result had to be positive for the final decision to be positive (i.e. both MT-UT and either MT-UT, respectively).

### 2.4 Reproductive management

After a voluntary waiting period of 50 days, the cows were enrolled in an oestrus synchronisation program (Flex-synch program). The cows in oestrus were artificially inseminated (AI) using frozen-thawed semen from bulls, in which normal fertility had been confirmed. After AI, the ovaries were examined daily by transrectal palpation until ovulation was confirmed. Delayed ovulation was diagnosed when ovulation had not occurred 24 h after the end of oestrus activity. When necessary, PGF$_2$α and GnRH were used to recover some of the reproductive dysfunctions, such as delayed ovulation, persistent corpus luteum, and developing cystic follicles. Conception was confirmed by detecting foetal heartbeat using ultrasonography at 35–40 days after each AI. If cows were not pregnant, the Flex-synch program was repeated. Calving difficulty, which was scored as 1 (no assistance), 2 (slightly assisted), 3 (assisted by two or three persons), 4 (assisted by four or more persons) or 5 (needed surgical treatment or death of dam), was evaluated (Martinez et al., 1983).

### 2.5 Statistical analysis

All statistical analyses were performed using JMP statistical software (JMP Pro Statistics and Graphics Guide, ver. 16. 1. 0; SAS Inst., Cary, NC). First, descriptive statistics were conducted for the reproductive performance of all cows, such as submission rate to AI, days to the first service, first service conception rate, conception rate until postpartum 100, 150 and 200 days, final conception rate, number of AI for pregnant cows and time to pregnancy.

The percentage of cows diagnosed as positive by the single and combined diagnostic criteria, and the proportion of cows with agreeing or mismatched results between MT and UT at 4 and 6 weeks postpartum were presented. A chi-square test was used to compare the frequency of the positive cows between 4 and 6 weeks postpartum.

The time to pregnancy was compared between the positive cows determined by each criterion and the true-negative cows that were determined as both MT-UT-negative. Survival curves were generated using Kaplan-Meier analysis. Curves of the positive cows determined by the single and combined diagnostic criteria were constructed relative to the true-negative cows, plotting the values of time to pregnancy against the proportion of cows in each group left to complete the calving conception period at each time point. The interval from calving to conception over 200 days was censored. Log-rank tests were used to compare survival curves. Finally, Cox’s proportional hazard models were used to assess the pregnancy rate per time until 200 days postpartum for the cows with MT-positive, UT-positive, both MT-UT-positive and either MT-UT-positive relative to the true-negative cows. The hazard ratios (HR) is constantly proportional and may be interpreted as the relative daily probability of pregnancy rate (the speed at which cows became pregnant) (Kasimanickam et al., 2004). For example, the HR = 0.5 means that the endometritis positive cows had a reduced pregnancy rate relative to the true-negative cows.

### Table 1

Descriptive analysis for reproductive performance of all 67 cows

| Items                                                      | Items                                                      | Items                                                      |
|------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------------|
| Submission rate to AI, % (n)                               | Days to first service, mean ± SD                           | First conception rate, % (n)                               |
| 97.0 (65)                                                  | 66.9 ± 15.5                                               | 12.3 (8)                                                   |
| Conception rate                                            |                                                            |                                                            |
| Until postpartum 100 days, % (n)                           | Until postpartum 150 days, % (n)                          | Until postpartum 200 days, % (n)                           |
| 21.5 (14)                                                  | 40.0 (26)                                                 | 60.0 (39)                                                 |
| Final conception rate, % (n)                               |                                                            |                                                            |
| 84.6 (55)                                                  |                                                            |                                                            |
| Number of AI for pregnant cows, mean ± SD                 |                                                            |                                                            |
| 3.4 ± 2.1                                                  |                                                            |                                                            |
| Time to pregnancy (days), mean ± SD                       |                                                            |                                                            |
| 158.2 ± 76.5                                               |                                                            |                                                            |

AI, artificial insemination.
TABLE 2  Percentage (number) of the cows with Metricheck device (MT) categories on a 0–5 scale at 4 and 6 weeks postpartum

| Week | 0   | 1   | 2   | 3   | 4   | 5   |
|------|-----|-----|-----|-----|-----|-----|
| 4    | 19.4 (13) | 46.2 (31) | 16.4 (11) | 14.9 (10) | 1.4 (1) | 1.4 (1) |
| 6    | 22.3 (15) | 47.7 (32) | 25.3 (17) | 2.9 (2) | 1.4 (1) | 0.0 (0) |

*0 = no discharge, 1 = clear mucus, 2 = flecks of purulent material within otherwise clear mucus, 3 = mucopurulent but < 50% purulent material, 4 = mucopurulent with > 50% purulent material and 5 = mucopurulent with > 50% purulent material and with an odour.

TABLE 3  Percentage (number) of the cows with ultrasonographic (UT) categories on a 0–2 scale at 4 and 6 weeks postpartum

| Week | 0   | 1   | 2   |
|------|-----|-----|-----|
| 4    | 55.2 (37) | 34.3 (23) | 10.4 (7) |
| 6    | 88.0 (59) | 8.9 (6) | 2.9 (2) |

*0 = anechogenic, 1 = echogenic compact contents, 2 = echogenic fluctuant contents.

A probability of \( p < 0.05 \) was considered significant, with probabilities between \( p \geq 0.05 \) and \( p < 0.10 \), indicating that a difference was approaching significance. Data are presented as the mean ± standard deviation (SD).

3 | RESULTS

3.1 | Description of the population

The reproductive performance of all 67 cows is shown in Table 1. Calving difficulty scores were recorded only for 53 out of a total of 67; thus, 32, 13, 4 and 4 cows had difficulty scores of 1, 2, 3 and 4, respectively.

3.2 | Diagnostic results determined by the single methods

Table 2 shows the percentage of the cows with MT categories on a 0–5 scale at 4 and 6 weeks postpartum. The distribution of the cows with each category was not significantly different between 4 and 6 weeks postpartum, and the number of cows with MT-positive results were similar at 4 and 6 weeks postpartum (34.3%, \( n = 23 \), and 29.9%, \( n = 20 \), respectively). Table 3 presents the percentage of the cows with UT categories on a 0–2 scale at 4 and 6 weeks postpartum. The number of cows with a 1 score decreased dramatically from 4 to 6 weeks postpartum; consequently, the percentage of cows with a UT-positive finding decreased dramatically from 44.8% \( (n = 30) \) to 11.9% \( (n = 8) \) from 4 to 6 weeks postpartum \( (p < 0.01) \).

Table 4 shows the score shift between 4 and 6 weeks postpartum for each MT and UT method. A greater number of the increasing score shift was detected when using MT compared with UT (22.4%, \( n = 15 \) vs. 3.0%, \( n = 2 \)). The percentage of the decreasing score shift was the same when using either MT or UT method (34.3%, \( n = 23 \)).

3.3 | Combination of the diagnostic result determined by MT and UT

Table 5 presents the combination of the diagnostic result determined by MT and UT at 4 and 6 weeks postpartum. The percentage of the cows with both MT-UT-negative; namely, the cows determined as the true negatives increased 20.9 points from 4 to 6 weeks postpartum. The percentage of the disagreements; namely, the cows with MT-negative and UT-positive results, conversely the cows with MT-positive and UT-negative results, were similar between 4 and 6 weeks postpartum (37.3% and 32.8%, respectively), but the former was greater than the latter at 4 weeks postpartum, and then the relationship reversed at 6 weeks postpartum. As the combined definition of two non-laboratory methods (MT and UT) was used, the number of cows with either MT-UT-positive was greater than that with both MT-UT-positive at 4 and 6 weeks postpartum. Additionally, there were the
**TABLE 5** Combination of the single diagnostic result determined by Metricheck device (MT) and ultrasonographic (UT) at 4 and 6 weeks postpartum

| Week | % (n) of diagnostic results determined by MT* and UT§ |
|------|--------------------------------------------------|
|      | Negative × Negative | Negative × Positive | Positive × Negative | Positive × Positive |
| 4    | 41.8 (28)           | 23.9 (16)            | 13.4 (9)            | 20.9 (14)           |
| 6    | 62.7 (42)           | 7.5 (5)              | 25.4 (17)           | 4.5 (3)             |

*MT-positive and -negative: the cows with Metricheck category at least 2 were defined as positive, and the others were defined as negative.
§UT-positive and -negative: the cows with ultrasonography category at least 1 were defined as positive, and the others were defined as negative.

**FIGURE 1** Survival curves for time to pregnancy up to 200 days postpartum for the positive cows determined by the single diagnostic method* and the true-negative cows at 4 and 6 weeks postpartum. *Diagnostic criteria at 4 and 6 weeks postpartum, MT-positive: cows with Metricheck category at least 2 were defined as MT-positive. UT-positive: cows with an ultrasonography category of at least 1 were defined as UT-positive. True-negatives: cows with both MT- and UT-negative. HR, Hazard ratio. CI, 95% confidence interval.

Cows sifted their diagnostic result from MT-negative to MT-positive from 4 and 6 weeks postpartum (14.9%, n = 10), while the cows sifted from negative to positive results was not detected when using the UT method.

### 3.4 Time to pregnancy

Figures 1 and 2 present survival curves of the positive cows diagnosed by the single and combined methods relative to the true-negative cows at 4 and 6 weeks postpartum, respectively.

Using the single methods at 4 weeks postpartum, survival analysis demonstrated that the proportion of non-pregnant cows in the positive cows diagnosed by MT tended to be significantly greater than that of the true-negative cows (Figure 1a). The hazard ratio of pregnancy until postpartum 200 days for the MT-positive cows tended to be significantly reduced relative to the true-negative cows. At 4 weeks postpartum, the proportion of non-pregnant cows was higher in the positive cows diagnosed by UT than in the true-negative cows, but no significant difference was detected (Figure 1c).

When using the combined methods, the proportions of non-pregnant cows in the positive cows diagnosed by both MT-UT were
significantly larger than that of the true-negative cows at 4 weeks postpartum (Figure 2a). The probability of pregnancy for both MT-UT-positive cows was significantly reduced relative to the true-negative cows. Similarly, at 6 weeks postpartum, the proportion of non-pregnant cows was higher in the positive cows diagnosed by both MT-UT, but no significant difference was observed (Figure 2b). At 4 weeks postpartum, the proportion of non-pregnant cows was higher in the positive cows diagnosed by either the MT-UT than in the true-negative cows, but no significant difference was detected (Figure 2c).

4 | DISCUSSION

Several reports have investigated the availability of single-use uterine diagnostic methods for predicting subsequent fertility. LeBlanc et al. (2002) suggested that cows with PVD between 20 and 33 days postpartum were 1.7-times more likely to be culled for reproductive failure than cows without PVD. Additionally, López-Helguera et al. (2012) indicated that cows with echogenic or anechogenic intrauterine fluid at 15 to 20 days postpartum were 11.1- or 7.7-times less likely to be pregnant at 70 days postpartum than cows lacking that, respectively.

In contrast, limited data are available on the efficiency of the combined use of non-laboratory methods to predict reproductive performance. Barlund et al. (2008) examined endometritis between 4 and 6 weeks postpartum and compared the efficiency of the combined non-laboratory technique, namely vaginoscopy, used to gather evidence of purulent or mucopurulent cervical discharge, and UT, which assesses uterine echogenic fluid. They suggested that the sensitivity to pregnancy status at 150 days postpartum was 16.4% for both positive vaginoscopy and UT, while those of the single usage were 7.1% and 10.0%, respectively. Lately, Šavc et al. (2016) investigated the availability of non-laboratory diagnostic methods, vaginal mucus scoring by MT, and assessment of uterine content by UT as predictors of future reproductive performance in dairy cows. They concluded that both prebreeding vaginal mucus evaluation with the MT and a UT scan of the uterus between 3 and 5 weeks postpartum served as good predictors of the subsequent pregnancy status, wherein the best predictor for the future reproductive performance of cows was a combination of both.

In the present study, the proportion of non-pregnant cows was significantly greater in both MT- and UT-positive cows than in the true negative cows. The hazard ratio of pregnancy for both MT- and
UT-positive cows was lower than that for MT-positive cows at 4 weeks postpartum (0.37 versus 0.51). Our results imply that a more severe effect on the chance of pregnancy was detected in cows diagnosed with both MT- and UT-positive compared with those diagnosed with MT- or UT-positive alone. While veterinary practitioners may consider single-use diagnostic tests as optimal, due to their rapid use in clinical practice, the combined use of non-laboratory methods is likely to provide veterinary practitioners with greater clinical support when diagnosing postpartum endometritis, which may improve the accuracy of predicting subsequent reproductive performance.

The diagnostic results of endometritis determined by non-laboratory methods often disagreed. For example, in our previous study, vaginal discharge containing pus was detected in 11.1%–23.0% of cows with the absence of intrauterine echogenic fluid; in contrast, intrauterine echogenic fluid was detected in 15.3%–42.8% of cows with the absence of vaginal discharge containing pus between 3 and 6 weeks postpartum (Kusaka et al., 2020). Similar disagreements were observed in the present study, as well as in previous studies (Šavc et al., 2016; Kelly et al., 2020). As our previous study indicated (Kusaka et al., 2020), these results may lead to confusion in veterinarians attempting to evaluate the uterine health status of cows under field conditions. Furthermore, 22.3% of the cows had an increasing MT score from 4 to 6 weeks postpartum, resulting in 14.9% of MT diagnostic results shifting from negative to positive between the two-time points in the present study. Similar to our results, McDougall et al. (2011) reported that 10% of the cows had the symptoms of endometritis getting worse from 4 to 6 weeks postpartum, evaluated by gross vaginal discharge score. Given these problems with the precision of diagnosis for endometritis, some previous studies suggested the importance of re-examination to determine the cow that needs treatment (Sheldon & Noakes, 1998).

The present study could not investigate whether the difference in the severity characterised by MT and UT score influence reproductive outcomes because our dataset was small. LeBlanc et al. (2002) and McDougall et al. (2007) indicated that the cows with purulent and foul vaginal discharge were associated with increasing impairment of the reproductive performance compared with the cows with mucus with flecks of pus in the vagina. To our knowledge, there was no study examining the association between the characteristics of intrauterine echogenicity and reproductive outcomes. To develop a more efficient and accurate diagnosis of endometritis, it is also needed to clear the association of the severity of endometritis characterised by MT and UT with reproductive performance. Furthermore, several risk factors are associated with uterine diseases, such as male calf, dystocia, parity, milk fever, ketosis, and metabolic disorders (Noakes et al., 2019). Dairy cows are typically under metabolic stress because they cannot consume enough food to meet the substantial extra demand for a nutrient that is required for lactation. The metabolic stress may compromise their immune system, which strongly contributes to the development of uterine disease. Because the present study did not include those potential risk factors in the analysis, the association of those factors with the MT and UT findings, resulting in reproductive outcomes, was not clear. To confirm the availability of the use of MT and UT, further research with larger animals in various herd management systems is required, taking into consideration the risk factors of endometritis.

5 CONCLUSION

The identification of both positive MT and UT was associated with a more serious undesirable effect on the chance of pregnancy than either positive MT or UT alone. The combined use of non-laboratory methods, namely MT and UT, could be recommended for veterinary practitioners to diagnose postpartum endometritis under field conditions, which could improve subsequently their reproductive performance.

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Hiromi Kusaka: Conceptualisation; data curation; formal analysis; investigation; methodology; project administration; supervision; writing – original draft. Taketoki Kimura: Investigation. Namiko Nishimoto: Investigation; resources. Minoru Sakaguchi: Conceptualisation; data curation; writing – review & editing.

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The Institutional Animal Care and Use Committee of the Kitasato University approved the experimental protocol.

DATA AVAILABILITY STATEMENT

Data available on request.

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ORCID

Hiromi Kusaka https://orcid.org/0000-0002-6745-5013
Minoru Sakaguchi https://orcid.org/0000-0002-0099-1583

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