Reliability and validity of a novel futsal special performance test designed to measure skills and anaerobic performance

Farhani, Farid; Rajabi, Hamid; Negaresh, Raoof ; Ali, Ajmol; Shalamzari, Sadegh Amani; Baker, Julien S.

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Reliability and Validity of a Novel Futsal Special Performance Test: Designed As a Skills and Anaerobic Performance test

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Authors: Farid Farhani¹*, Hamid Rajabi¹, Raoof Negares⁵, Ajmol Ali³, Sadegh Amani Shalamzari¹, Julien S Baker⁴

Affiliation:
1 Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, Kharazmi University, Tehran, Iran
2 Department of Physical Education & Sport Sciences, Faculty of Humanities, Tarbiat Modares University, Tehran, Iran
3 School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand
4 Applied Physiology Research Laboratory, School of Health and Life Sciences, University of the West of Scotland, Lanarkshire Campus, Scotland, UK

Corresponding author: Farid Farhani
Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, Kharazmi University, Tehran, Iran.
Phone: +989359382091
E-mail: F.Farid1993@yahoo.com

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Reliability and Validity of a Novel Futsal Special Performance Test: Designed As a Skills and Anaerobic Performance test

Abstract

Purpose: This study examined the validity and reliability of a novel futsal special performance test (FSPT) as a measure of futsal performance and skills. Methods: Thirty six futsal players with different levels of experience were recruited and divided into two groups (elite and non-elite). Players participated in four sessions (at least 7 days apart); a) familiarization session, b) anaerobic power (Wingate test), c) FSPT trial 1, and d) FSPT trial 2. The FSPT was carried out on a futsal court (wooden sprung floor) and examined skills such as dribbling, rotation, long and short passing and shooting. Content validity was assessed using 6 experienced futsal coaches and instructors. Results: There was a significant correlation between FSPT and various aspects of anaerobic power ($r=0.5$ to $0.91$, $p\leq0.001$). Moreover, significant large correlations were observed between test and re-test of FSPT ($r=0.77$; 95% confidence intervals (CI)$=0.56$ to $0.98$; $p\leq0.001$). All instructors and coaches confirmed the content validity. There was high inter-rater reliability of the FSPT ($r=0.89$; 95% CI$=0.85$ to $0.93$; $p<0.001$). FSPT total ($p=0.001$), penalty ($p=0.022$) and performance ($p=0.001$) time was superior in elite relative to non-elite players. Anaerobic power was greater in elite players ($p<0.001$). Conclusion: Our results support the use of the FSPT to assess futsal players’ performance in conjunction with skill and anaerobic fitness.

Keywords: FSPT, Futsal skills, Dribbling, Passing, Anaerobic power
Introduction

Futsal is the 5-a-side version of soccer, played in a smaller area than a football pitch (40 m length and 20 m wide) and typically played indoors. Futsal consists of intermittent high-intensity exercise activities, that change more often than soccer (every ~3.2 s), resulting in higher agility and sprint running performance, but lower vertical jump and half-squat power performance than soccer. The ratio of activity to rest in futsal is about 1:1, and although there is a high anaerobic demand, more than 75% of all energy is resynthesized by the oxidative phosphorylation pathway during match play. Previous analysis estimated that professional futsal players perform at a high intensity (>80% VO\textsubscript{2max}) which consists of 46% of total game distance or time. Although there is some research on physiological demands of futsal including agility and high-intensity running, investigations into futsal skill performance which include shooting and dribbling are rare, and thus may hinder coaches’ ability to optimize training.

Furthermore, current futsal tests may have some limitations such as examination of one parameter (i.e., aerobic fitness). There may also be limitations in evaluating the skills associated with the game which include focusing only on one skill (i.e., pass). Although, tests such as the Futsal Intermittent Endurance Test (FIET), Yo-Yo, Hoff and Massey Futsal Shooting Test (MFST) are designed and used to determine the level of fitness in soccer and futsal, these tests are not specific to futsal, only consider one aspect of futsal (i.e. shooting), or are more applicable to soccer. Therefore, it seems that previous tests are general and not wholly representative of futsal. The popularity of futsal is rising, there is specificity of motor patterns and there are different demands to those of soccer performance but there is a lack of studies reporting a special, valid and reliable holistic futsal test. Therefore, the purpose of this study was to design a futsal-specific test, which consists of evaluation of futsal skills (dribbling, dribble, long pass,
short pass, ball control, rotation, combined movement, shoot and return from attack to
defence), and assess its validity and reliability, in Iranian futsal players. Futsal is one of
the most popular sports in Iran and Iran has been ranked among the top 10 teams in the
world. Moreover, most Iranian professional soccer players started with futsal prior to
playing soccer. As the assessment of anaerobic power is useful to select players for
optimal performance, a further objective was to quantify measures of the error rate and
establish anaerobic power (i.e., peak power) profiles of the players.

Methods

Participants

Thirty-six healthy, male, outfield futsal players, with at least 4 years’ experience,
volunteered to participate in the study. Of the participants that were eligible for the study,
18 players were elite futsal players and 18 players were non-elite futsal players (Table 1).
Elite players were defined as those playing for the national team or played in the Iranian
Golden League. Non-elite players were categorized if they played in the second or third
division of the Iranian futsal league, or players who were physical education and sports
science students. Following examination by the physician, to establish the health status of
the participants, all risks and benefits of the study were explained to players. All
participants then signed written informed consent forms. The study was approved by the
Ethics Committee of the Institute of Physical Education and Sport Sciences of Iran
(IR.SSRI.REC.1396.187).

Research design
Participants were invited to a preliminary session within the exercise physiology laboratory of Kharazmi University to explain the objectives and process of the research and to collect demographic and anthropometric data. Players participated in three other sessions which were separated by seven days; in session one, the Wingate anaerobic test was performed; and in both sessions two and three, the FSPT was undertaken. The temperature and humidity of the research site were kept constant between 18-21 °C and 50-65%, respectively. All tests were carried out between 4:00 and 6:00 pm. The study was performed 3 weeks after completing the in-season futsal period. This ensured that the players were fully recovered from the effects of prior matches and tournaments. The last high-load session of exercise training was during in-season period; no match was held after completing the in-season futsal period. During these three post-season weeks, routine exercise sessions mainly consisting of technical and tactical tasks were performed. The last meal was consumed 3 to 4 hours before the test session; the same meal was used. After this meal and during the test sessions participants did not consume any food and only water was allowed.

For the evaluation of anaerobic power, players were required to complete a 30-s anaerobic Wingate test (Monark 894E, Sweden). For FSPT, coloured cones and an official futsal ball (Star No. 4, FB524-05) was used. Players were advised not to take any supplements (including caffeine and creatine) during the study period.

During FSPT, two referees blinded to the group allocation (elite vs. non-elite) separately recorded the errors and the total time of the test. Additionally, two AFC instructors, one international instructor in exercise science and conditioning, two futsal instructors, and two futsal coaches were recruited to comment on the content (or face) validity of the FSPT.
**Futsal Special Performance Test**

Before performing FSPT, all players were involved in 15 min running, sprints, and small-sided games as a warm-up. As shown in Fig. 1, cone location included 14 orange cones, 1 purple (start cone) and 1 green (end cone) cone. Four elite futsal players (not from participants) and two referees were also recruited and located as shown in Fig. 1.

The testing participant takes position behind the start cone (purple cone). After the start whistle, the participant was required to run with the ball 8 m (from A to B cone; step 1) and then dribble zigzag with the ball through 7 orange cones (B to H cones; step 2). After turning past cone I, the participant sends a long pass to the first passing player (PP) next cones K, and then proceeds to the location of cone J (step 3). The first PP passes to the participant and participant returns the ball (step 4). Then, the participant moves to location cone L, then, receives and returns the ball to the second PP (step 5). The participant then repeats this step again, and after receiving the ball (for the third time), he rotates and dribbles (step 6), performs a wall pass with the player next to the cone N (step 7). After receiving ball in landing location 1, the player shoots the ball with maximum effort (step 8). Finally, the participant proceeds to the ball landing location 2 and receives a long pass and shoots at the goal (step 9). He then moves to the final cone (green cone).

The test is completed when the participant crosses the end green cone. The participants are encouraged to perform the test with maximum speed and power. Time was calculated using manual chronometers by two referees and the mean of the two values was recorded. The penalty time of errors when performing the test was also recorded; these included:

- ball hitting cone: 2 s
- wrong pass: 2 s
- no goal and completely missing the goal framework: 2 s
- ball hitting goal framework: 1 s

Performance time was obtained by adding the time to complete the test (”time only”) with penalty time.

**Wingate Test**

To measure anaerobic power, players performed a 30 s Wingate cycling test. At the preliminary session, athletes became familiar with performing the Wingate test. Prior to the test, participants were seated on the cycle that was calibrated (seat height, seat position, handle bar position, and handle bar height) for optimal comfort and pedaling efficiency. In the warm-up protocol, athletes cycled 5 min with light cycling resistance and sprint cycling for 5 s at the end of every consecutive minute. After 2 min of active recovery, athletes performed 15 s of acceleration at 70 rpm with work resistance set at 0.025 kg per kg body mass. Afterwards the full load was used with frictional resistance at 0.075 kg per kg body mass for each participant. To calculate the relative power (w/kg), absolute power (w) was divided by the player’s weight (kg). All players were familiarized to testing conditions prior to data capture, and all testing was performed at the same time of day.

**Statistical Analysis**

To determine construct validity, the difference between elite and non-elite players was measured using independent t-test; and the non-parametric, Mann-Whitney U was used when parametric assumption (i.e., normality) was not fulfilled. Pearson’s correlation test was used to investigate relationships between variables, and for ordinal variables, Spearman’s correlation was used. To determine the reliability, Pearson correlation and
intraclass correlation coefficient (ICC) was performed between test-retest. Also, Pearson correlations were taken between Wingate test parameters and FSPT (time only and time performance) and <0.3, 0.3-0.5, 0.5-0.7 and >0.7 were considered very small, small, moderate and large correlation, respectively. All mentioned analyses were performed with SPSS 21; significance was defined as p<0.05. Cohen’s d (effect size) values were calculated and <0.20, 0.20-0.50, 0.51-0.80 and >0.80 were considered trivial, small, moderate, and large effects, respectively. The r, effect size and ICC were accompanied with 95% confidence intervals (CI). Furthermore, a median-split table was used to assess criterion validity. This method examines the number of players in the “expected” group (elite vs. non-elite group) based on median-split values.

Results

There were no differences in age (p=0.481), height (p=0.627), weight (p=0.567), and BMI (p=0.405) between elite and non-elite groups (Table 1). FSPT results indicated that time only (p=0.001; d=3.42, large effect), penalty time (p=0.022; d=0.88, large effect) and performance time (p=0.001 and d=3.19, large effect) of the elite players was superior compared with non-elite players (Table 2). Elite players also showed higher relative peak power (p= 0.001; d=0.95, large effect), relative average power (p= 0.001; d=1.10, large effect) and minimum power (p= 0.001; d=1.38, large effect) and lower fatigue index (p= 0.001 and d=1.44, large effect) in the Wingate test (Table 2).

FSPT time only and performance time showed significant correlations with all of the Wingate test variables (r=-0.52 to r=-0.91, p<0.05, for measures of power; and r=0.50 to r=0.75, p<0.05, for fatigue index; Fig 2).

The results of the median-split analysis (Table 3) showed that both time only and performance time of FSPT were different between elite and non-elite players; all players
for time only and 16 of 18 players in each elite and non-elite group for performance time were in the “expected” group. In addition, for penalty time, 10 non-elite and 8 elite players of the 16 players were in the “expected” group based on median-split values; 6 and 4 of 16 players were equal with the median from elite and non-elite players, respectively.

There were no differences between FSPT trial 1 and trial 2 for time only (30.48±2.29 s vs. 29.91±2.27 s; p=0.28), penalty time (2.27±1.78 s vs. 1.58±1.18 s; p=0.11) or performance time (32.76±3.18 s vs. 31.49±2.97 s, p=0.08). Moreover, significant relationship and ICC were also observed between test re-test for FSPT for time only (r=0.77, 95% CI=0.56 to 0.98; ICC=0.75, 95% CI=0.55 to 0.86), penalty (r=0.59, 95% CI=0.38 to 0.80; ICC=0.37, 95% CI=0.06 to 0.61) and performance time (r=0.75, 95% CI=0.69 to 0.81; ICC=0.70, 95% CI=0.45 to 0.84) time (all p≤0.05; Fig 3).

There was a significant correlation between both referees’ sets of scores for total time only (r=0.89, 95% CI=0.85 to 0.93; ICC=0.86, 95% CI=0.79 to 0.94; p<0.001) and performance time (r=0.94, 95% CI=0.91 to 0.97; ICC=0.94, 95% CI=0.88 to 0.97; p<0.001) of FSPT.

Discussion

The purpose of this study was to design a special futsal test that included the skills (pass, dribbling and shooting) and abilities (speed and agility) which are typically used in futsal. Our results showed that the designed test had an acceptable validity and reliability and, given that the test was designed on a futsal pitch and used futsal skills, it complied with the specificity principle.

Since that anaerobic power can predict performance of the futsal players in decisive moments of the match (i.e., scoring a goal), the 30-s Wingate test was selected...
as one of the most authoritative anaerobic power test for investigating the relationship between FSPT data and anaerobic power. We used bivariate correlational analysis and showed that peak power, average power, minimum power and fatigue index were all significantly correlated with both total time only and performance time (moderate to strong correlation). Interestingly, lower $r$ value which observed for performance time when compared with total time only probably related to the simplicity of the test. Therefore, FSPT time only may be better related with anaerobic power determined by Wingate test of futsal players, at least when compared to values from the Wingate test.

The Wingate measures anaerobic power and has a similar energy system definition, supply and demand to the test.\(^{21}\) In addition, total time of the FSPT often ranged between 26 to 34 s, which is similar to Wingate test time. As demonstrated in Table 3, we used median-split method to assess criterion validity.\(^{24}\) Data revealed that for performance and time only, nearly all of the players fell into their respective location; thus both mentioned items have high criterion validity. Additionally, however, we observed no clear penalty time differences in ranking for points scored, but elite players’ were had a trend to be below median that strengthens the test validity. Moreover, evidence suggested that anaerobic power indicators such as blood lactate are higher during a specific vs. non-specific intermittent test\(^{25}\) such as the current test. Therefore, a test design that has the specificity to mimic actual performance conditions is important and is probably a better indicator of performance and power than general performance tests.

To assess content validity, we recruited instructors and futsal coaches as previously mentioned. All individuals approved that this test is a valid tool to screen futsal players performance as well as abilities and skills.

Test and re-test results demonstrated a strong correlation for total time only and performance time, with only moderate correlation for penalty time. The 30% reduction in
the penalty time (from 2.27±1.78 s to 1.58±1.18 s), which probably indicated a learning effect, is one possible interpretation for the moderate correlation between two trials for penalty time. In fact, the penalty time was two-fold higher in non-elite vs. elite players. Furthermore, the learning effect was higher in the non-elite players, so that penalty time in the non-elite players reduces 0.88 s in second trial while increased 0.05 s in elite players. The reason for the higher penalty time reduction and the learning effect in the non-elite players can be related to the level of skill and experiences gained from more training years. Additionally, total time only and performance time reduced less than 4%. Nevertheless, the overall results strongly support test reliability when the test was repeated.

Hopkins suggests that in performance tests, the smallest worthwhile enhancement can be calculated as 0.2 of the between-participants standard deviation. In FSPT, the mean of the total time only and performance time of participants was 30.48 s and 32.76 s, respectively, and the smallest worthwhile effect (i.e. sensitivity of test) was 0.45 s and 0.63 s which represent 1.47% and 1.94%, respectively. In addition, sub-group results (elite vs. non-elite) examination demonstrated that elite players have 14% and 18% better performance values for the total time only and performance time of the test. Collectively, it seems that futsal players that have a superior ability and skills performed better in FSPT thus supporting the high construct validity of the test. Elite players showed 48% less penalty time, completed the test quicker and thus achieved much better total and performance time (Table 2); this means that elite players succeeded in performing the test using the least time (and possibly energy) and without sacrificing movement speed and technique accuracy. However, non-elite players were slower (recorded higher time) in an attempt to maintain their accuracy and accumulate less error. This method helped to
distinguish skill levels between groups and increase the construct validity of the performance time as shown in previous studies.\(^7\)

The referees’ contribution to FSPT was important as they recorded total time only and observed penalties during the test. Results recorded by referees were the same; and correlations indicated a strong relationship, indicating good inter-rater reliability in terms of detection of penalties accrued.

Comparing current results with previous studies and tests is difficult because of different protocols used and test specificity. A study conducted by Ali et al., used skill rather than technique for measuring skill in soccer.\(^24\) A further study conducted by Castagna et al., examined the FIET and showed that the FIET measures the energy system of futsal. The FIET was without the ball and did not measure or use futsal skill.\(^5\) However, the FSPT includes four skills used in futsal which distinguishes this test from other tests.\(^24,28–31\)

**Limitations and future research**

The current test was only performed by male futsal players; therefore, further studies are needed to investigate the validity and reliability of FSPT in female players and younger players. The sample size of the current study is 36 futsal players, 18 elite and 18 non-elite. Future studies can examine FSPT in larger cohort in order to obtain a more accurate validity and reliability. Repeating FSPT during a season may demonstrate the sensitivity of the test to a training period and capture changes during the season. Finally, futsal has a variety of physiological demand, such as agility, endurance and muscle coordination,\(^5\) but the current study did not investigate the correlation of FSPT data with performance related to these demands. Another limitation is the human error of the players who help to performing the test include passing players. However, in this test,
professional futsal players have been used as passing players (the same players for all participants), but individual skills and the variability of the pass during the test and the implications for the reproducibility of the test in the real scenarios is another effective limitation.

**Practical Applications**

Our results support the use of the FSPT to assess futsal players’ anaerobic fitness. The FSPT test is a simple and practical tool for coaches and instructors, because it does not require expensive or special equipment. Moreover, the current test is appropriate to investigate the differences between performance according to player level (elite and non-elite). In addition, this test has potential training applications, because the test can be used as a practical intermittent and power training exercise using the ball.

**Conclusions**

Our results suggest that FSPT is a valid and reliable test to assess the skill aspects related to futsal. Moreover, the FSPT is a sensitive test to differentiate futsal performance according to playing ability in our cohort of players. In addition, results indicated that the FSPT has high construct validity. Finally, further studies are required to investigate other aspects of this test and its relationship with different parameters of match-performance such as aerobic capacity and performance agility.

**Acknowledgments**

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References


Figure 1. Spacing, cones and players location and test procedure. Sixteen cones located as shown in above figure. After warm-up, participant was located behind the purple cone. In step 1 and 2, participant runs with the ball and dribble. Afterwards, turn cone I, sends a long pass, and goes near cone J (step 3). After repetition step 4 and 5 include receiving and sending short pass, participant rotates and dribbles; then performs a wall pass and shoot the ball to the goal. Finally, in step 9, participant receives long pass and shoot to the goal, and subsequently goes to the final cone. Time was calculated by two referees and their records average was considered as total time. The penalty time was also recorded during the test (refer to the text), and performance time is obtained by adding penalty time to the total time.
Figure 2. Pearson correlation analysis between total and performance time of FSPT with power indicators. There are significant correlation between absolute and relative peak power (a, b, g and h), average power (c, d, i and j), minimum power (e, f, k and l) and fatigue index (m and n) with total and performance time of FSPT ($p<0.01$).
Figure 3. Test and re-test results of total (a) and performance (b) time of FSPT. All analysis revealed a significant correlation ($p<0.01$).
Total time only

Performance time

201x82mm (300 x 300 DPI)
Table 1. Anthropometric characteristics of elite and non-elite Futsal players

<table>
<thead>
<tr>
<th></th>
<th>age (years)</th>
<th>height (cm)</th>
<th>weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Experience (years of play)</th>
<th>Number of training sessions per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elite (n=18)</td>
<td>23.0 ± 1.83</td>
<td>175.0 ± 0.05</td>
<td>67.2 ± 4.74</td>
<td>21.9 ± 1.79</td>
<td>7.5 ± 2.79</td>
<td>5.9 ± 1.39</td>
</tr>
<tr>
<td>Non-elite (n=18)</td>
<td>22.6 ± 1.41</td>
<td>174.0 ± 0.04</td>
<td>68.2 ± 6.11</td>
<td>22.4 ± 1.80</td>
<td>6.6 ± 2.65</td>
<td>5.2 ± 1.52</td>
</tr>
<tr>
<td>Total (n=36)</td>
<td>22.8 ± 1.62</td>
<td>174.1 ± 73.17</td>
<td>67.67 ± 5.42</td>
<td>22.2 ± 1.79</td>
<td>7.0 ± 2.72</td>
<td>5.6 ± 1.47</td>
</tr>
</tbody>
</table>
Table 2. Wingate Test variables, special Futsal performance test time and penalty of elite and non-elite Futsal players

<table>
<thead>
<tr>
<th>Variable</th>
<th>All players (n = 36)</th>
<th>Elite (n = 18)</th>
<th>Non-elite (n = 18)</th>
<th>t value</th>
<th>P value</th>
<th>Effect size</th>
<th>95% CI of Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak power</td>
<td>W</td>
<td>742.4 ± 44.0</td>
<td>770.2 ± 34.6</td>
<td>4.864</td>
<td>0.000*</td>
<td>1.62</td>
<td>0.87 – 2.37</td>
</tr>
<tr>
<td></td>
<td>W/Kg</td>
<td>11.0 ± 1.1</td>
<td>11.5 ± 1.1</td>
<td>2.693</td>
<td>0.011*</td>
<td>0.95</td>
<td>0.26 – 1.64</td>
</tr>
<tr>
<td>Average power</td>
<td>W</td>
<td>627.1 ± 43.9</td>
<td>655.2 ± 36.6</td>
<td>4.962</td>
<td>0.000*</td>
<td>1.65</td>
<td>0.9 – 2.41</td>
</tr>
<tr>
<td></td>
<td>W/Kg</td>
<td>9.3 ± 1.0</td>
<td>9.8 ± 1.0</td>
<td>2.976</td>
<td>0.005*</td>
<td>1.10</td>
<td>0.40 – 1.80</td>
</tr>
<tr>
<td>Minimum power</td>
<td>W</td>
<td>405.7 ± 40.9</td>
<td>432.8 ± 32.6</td>
<td>5.263</td>
<td>0.000*</td>
<td>1.75</td>
<td>0.99 – 2.52</td>
</tr>
<tr>
<td></td>
<td>W/Kg</td>
<td>6.0 ± 0.8</td>
<td>6.4 ± 0.7</td>
<td>3.719</td>
<td>0.001*</td>
<td>1.38</td>
<td>0.65 – 2.10</td>
</tr>
<tr>
<td>Fatigue index</td>
<td>%</td>
<td>45.4 ± 2.5</td>
<td>43.8 ± 2.3</td>
<td>-4.722</td>
<td>0.000*</td>
<td>1.44</td>
<td>0.71 – 2.17</td>
</tr>
<tr>
<td>Total time only</td>
<td>s</td>
<td>30.48 ± 2.29</td>
<td>28.52 ± 1.36</td>
<td>-10.195</td>
<td>0.000*</td>
<td>3.42</td>
<td>2.39 – 4.44</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>2.27 ± 1.78</td>
<td>1.55 ± 1.38</td>
<td>90.000*</td>
<td>0.022*</td>
<td>0.88</td>
<td>0.19 – 1.56</td>
</tr>
<tr>
<td>Performance time</td>
<td>s</td>
<td>32.76 ± 3.18</td>
<td>30.08 ± 1.77</td>
<td>-9.553</td>
<td>0.000*</td>
<td>3.19</td>
<td>2.20 – 4.17</td>
</tr>
</tbody>
</table>

* denotes a significant difference between elite and non-elite players (p<0.05), #: Penalty time is an ordinal variable and nonparametric test was used to measure the difference between elite and non-elite players.
Table 3. Median-split table for special Futsal performance test between elite and non-elite players

<table>
<thead>
<tr>
<th></th>
<th>Above median</th>
<th>Below median</th>
<th>Equal median</th>
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<tbody>
<tr>
<td><strong>Total time only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elite</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Non-elite</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Penalty time</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elite</td>
<td>4</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Non-elite</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Performance time</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elite</td>
<td>2</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Non-elite</td>
<td>16</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
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Dear Prof. Dr. Karim Chamari;

Thank you for reviewing our revised manuscript, titled “Reliability and Validity of a Novel Futsal Special Performance Test: Designed as a Skills and Anaerobic Performance test”. We are grateful for the additional constructive comments provided by the reviewers, and the resulting revisions further improved the clarity and contribution of our manuscript. Based on the input from the reviewers, the manuscript was revised as described below, and the changes within the manuscript are colored in red type.

Note: We, according to the editor comment, have made changes (in red) on the use of the "anaerobic" in throughout the manuscript. As for the title, since the reviewers emphasized in his previous comments that there should be an “anaerobic” emphasis on the title, the word “anaerobic” is included.

Reviewer: 1
Comments to the Author
The authors made major changes throughout the article. Many of the suggestions were attended and the manuscript was improved.

Some minor suggestions:
Results: "high effect" it is not included in the magnitude inference. It must be re-written (or trivial, or small, or moderate, or large or very large).
Response to reviewer: Thank you for your comments. We have modified the text and we used "large" instead of "high".

A major concern: the variability of the pass during the test and the implications for the reproducibility of the test in the real scenarios.
Response to reviewer: Thank you for your comment. We agree with your comment. The “variability of the pass during the test” is an important limitation of our designed test. Therefore, we explained this in the discussion section (Limitations and future research). Please see Page 11, paragraph 4 and Page 12, paragraph 1.