Analysis of time exposure to DNA touch quality on face shield using STR CODIS – TH01 and D18S51

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Abstract: A number of countries, including Indonesia, have taken preventive measures and made efforts to break the chain of the spread of COVID-19. The impact of government policies is felt in the economic sector, which causes many people to lose their jobs but is required to meet the needs of daily life. This affects the increase in crime rates during the pandemic, which coincides with the government's policy to implement health protocols by using face shields outside the home. This allows the discovery of evidence in the form of a face shield to be identified through DNA touch. This type of research is experimental analytic with a time-series design. This study aims to analyze the effect of exposure to the environmental temperature range of 28.2 °C - 29.3 °C and humidity range of 88% - 94.2% on the quality of DNA touch on the face shield by giving the 1st, 7th, and 14th-day duration treatment using STR CODIS. The result is an effect of prolonged exposure time on DNA quality, as evidenced by the Anova test with p < 0.05.

Keywords: Crime; DNA Touch; DNA Quality; Face Shield.

INTRODUCTION

The government has taken preventive measures and efforts to break the COVID-19 transmission chain, some of them are social activity restrictions such as working from home, studying from home, maintaining distance, using personal protective equipment (masks and face shields), and washing hands.1 Government's policies that are enforced might trigger an economic crisis, which causes many people to lose their jobs.2,3 Mobility restrictions and changes lead to increased crime rates because many people have lost their jobs but still have to make their ends meet.4 Crimes during the pandemic include theft, looting mini-markets, robbery, and mugging.5 The increase in more time spent at home during the pandemic also triggers violent crimes such as domestic violence and child abuse.4 Another crime that takes advantage of the pandemic is when the government and security forces are focusing on handling Covid-19, namely terrorism. It is easier for terrorists to carry out their acts of terror, such as the suicide bombing that occurred at the Makasar Cathedral Church in March 2021.
These crimes occurred during the pandemic period, which coincided with the issuance of a government policy to implement a health protocol by using a face shield when outside the home in the form of a face shield, this made it possible to find evidence in the form of a face shield at the crime scene or its surroundings. Identification can be made with face shield evidence by examining DNA in the form of DNA touch originating from skin and sweat epithelium. DNA touch, which is DNA transfer due to contact from the perpetrator or victim with surrounding objects that are at the crime scene (TKP), can increase the potential for trace evidence and make it easier for investigators to relate it to the facts of the incident and prove the perpetrator or victim to avoid proof errors. A previous study has analyzed DNA quality on exposure to high temperatures ranging from 500°C - 750°C and on the effect of prolonged exposure on DNA quality to determine gender. A study on identifying evidence using DNA touch on a face shield has not yet been carried out, while one of the potential pieces of evidence during a pandemic that can be found at a crime scene or its surroundings can be a face shield. Findings of evidence are not always found at the time of the incident but can also be found a few days after the incident. Evidence in the form of DNA, especially DNA touch, is easy to degrade and decrease in quality, one of which is caused by temperature. Therefore, the purpose of this study is to determine the effect of environmental temperature on the quality of DNA touch on a face shield that is still effective, which can be used as forensic identification material in determining perpetrators and victims by utilizing traces of evidence in the form of DNA touch.

MATERIAL AND METHOD

The study population was 15 student community volunteers. Volunteers who were used as the study population had met several research criteria such as being male, aged 15-30 years, being active outdoors for 3 hours, and being willing to fill out informed consent. The sample size was calculated using the Cochran and Cox method with SD = 13, the lowest mean = 120.17, and the highest mean = 144.67 obtained 7 repetitions for each sample treatment group. So the required sample is 21 samples obtained from the results of the repetition calculation multiplied by the number of treatments (7 x 3 = 21 samples). Sampling was carried out by random sampling; Fifteen volunteers were then randomly selected by asking them to draw a paper containing numbers from 1 to 7, where people who picked the paper that contained the numbers were selected as respondents.

The DNA touch sample collection was obtained by asking the respondent to use a new face shield that had not been opened from the wrapper, and then the face shield was used for 3 hours while doing outdoor activities. The samples were then taken to the Human Genetic Institute of Tropical Disease Laboratory, Universitas Airlangga, for cutting. The cuts were made in 3 parts for each face shield sample. The total number of samples was 21 pieces of a face shield, divided into 3 groups according to different exposure times at ambient temperature. Group I consisted of 7 samples exposed to ambient temperature for one day, Group II consisted of 7, which were exposed to ambient temperature for seven days, and Group III consisted of 7 exposed to ambient temperature for 14 days. We selected 1, 7, and 14 days as exposure time because one-day exposure is correlated to the beginning of the crime scene identification process by the investigator (first day), the 7th day is the maximum limit for investigators to
conduct a crime scene examination, and the 14th day is the limit for submitting case files to the public prosecutor.\textsuperscript{13,14}

Group I DNA samples was extracted using DNAzol with the initial steps: The DNA extraction stage used the DNAzol method by immersing the face shield pieces in 20 cc sterile distilled water in a 50 cc conical tube sonicated for 15 minutes. The sonicated results were transferred to another tube and centrifuged at 12000 rpm for 10 minutes to collect the pellets. The resulting pellet was added with 1000 l of phenol and 200 l of chloroform and then incubated for 2 hours. The sample was centrifuged at 8000 rpm for 10 minutes, added isopropanol ratio (1:1), and incubated for 1 hour. The sample was centrifuged again at 12000 rpm for 10 minutes to obtain DNA on the pellet. The pellets were then washed with 70% ethanol and then centrifuged. The supernatant from the centrifuge was discarded by inverting the tube and placing it in an upright position until the ethanol evaporated and dried, then 50 l sterile distilled water was added and stored at 40C. The same was done for the samples on the 7th and 14th days. After all the samples were extracted, it was continued to determine the content and purity of the DNA using a UV-Vis spectrophotometer at a wavelength of 260 – 280 nm. Then, DNA amplification was carried out by PCR using the TH01 and D18S51 loci, and the last step was electrophoresis using acrylamide gel to see the DNA band against the TH01 (152 – 195 bp) and D18S51 (286 – 366 bp) loci.

The obtained data was twenty-one DNA content from the spectrophotometer result, then statistically analyzed using SPSS software version 23. The study data was first tested with the Shapiro Wilk normality test with $\alpha = 5\%$ and homogeneity test, where a p-value of > 0.05 revealed that the obtained data were normally distributed and homogeneous respectively. The next step was to perform the statistical test using the One Way ANOVA Test.

**RESULTS AND DISCUSSION**

The results of DNA level measurements (Table 1) were conducted using a spectrophotometer at a wavelength of 260/280 nm. The table shows different mean DNA touch levels and purity on faces shields on each exposure time (1, 7, and 14 days) and the mean ambient temperature and humidity. The minimum requirement for DNA purity to have proceeded to DNA amplification is 1.8 – 2, while the Short Tandem Repeat (STR) examination requires a minimum DNA level of 1 – 25 ng. The results of DNA level and purity measurement of this study had reached the minimum required DNA.\textsuperscript{15}

<table>
<thead>
<tr>
<th>Time Exposure</th>
<th>Sample Number</th>
<th>The average amount of DNA (X±SD) (µg/ml)</th>
<th>Average Purity of DNA</th>
<th>Average Ambient Temperature (°C)</th>
<th>Average Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>7</td>
<td>1778±529.30</td>
<td>1.29</td>
<td>28.6</td>
<td>90</td>
</tr>
<tr>
<td>Day 7</td>
<td>7</td>
<td>856±258.55</td>
<td>1.22</td>
<td>28.9</td>
<td>89</td>
</tr>
<tr>
<td>Day 14</td>
<td>7</td>
<td>1153±487.58</td>
<td>1.24</td>
<td>28.7</td>
<td>89.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>1550404.333</td>
<td>7.954</td>
<td>0.003</td>
</tr>
<tr>
<td>Within Groups</td>
<td>18</td>
<td>194918.889</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 2 show differences in DNA quality which was affected by exposure time (1, 7, and 14 days). The difference in DNA quality level could be seen in table 1, while the fluctuations that occurred could be seen in figure 1. Figure 1 provides an overview of fluctuations in DNA levels at different exposure...
times. The decrease in DNA level occurred on day 7 and then increased on day 14.

Figure 1. Graph of Mean DNA Touch Level on Face Shield Based on Different Exposure Days

Figure 2 is a visualized image using acrylamide gel at the Short Tandem Repeat Combined DNA Index System (STR CODIS) TH01 (119 – 155 bp) locus of DNA touch samples at the highest and lowest levels, while figure 3 is the result of visualization of STR CODIS D18S51 (286 – 366 bp) at the highest and lowest levels. The visualization of STR CODIS TH01 and D18S51 are the finding of bands in all samples. This indicates that the selected loci is a potential locus for the respondents in accordance with the recommendations of the FBI (Federal Bureau Investigation) and is an illustration of the success of the amplification process. Good visualization during electrophoresis indicates that the purity and content of DNA obtained are adequate to be used as a DNA examination material. In addition, the DNA obtained should not be degraded, if it undergoes degradation, it must be kept to a minimum because it causes the primer not to attach during the annealing process to the target DNA to be amplified. The difference in the fluctuation of DNA touch levels from the results of the study on the length of time of exposure still gives good results in DNA visualization; this proves that the sample in this study produced good levels and purity of DNA.

Figure 2. Visualization of electrophoresis results at the TH01 locus (152 - 195 bp) on day 1, day 7, and day 14

**Description:**
- E1 : Day 1 Sample (highest DNA level)
- F7 : Day 7 Sample (highest DNA level)
- E14 : Day 14 Sample (highest DNA level)
- F1 : Day 1 Sample (lowest DNA level)
- E7 : Day 7 Sample (lowest DNA level)
- B14 : Day 14 Sample (lowest DNA level)
- M : Marker ladder 100 bp
Factors that can influence differences in DNA quality and fluctuations are due to DNA touch derived from skin epithelial cells (corneocytes), sweat, protein, free DNA, and contamination from the environment. Touch DNA derived from corneocytes is considered to have little DNA content and is easily fragmented, but skin epithelial cells still contain residual DNA that can provide value for identification using short tandem repeats (STR). The difference in DNA touch level was influenced by the tendency of a person's ability to leave DNA touch, the surface used as attachment location, and the exposure time of DNA to external factors.

Exposure time, temperature, humidity, UV ray, and microorganism are factors that trigger DNA degradation, which could affect the difference in DNA quality.

DNA degradation is influenced by environmental factors (endogenous factors), including humidity, temperature, UV light, and microorganisms. The DNA degradation affects the primer attachment process to target DNA, which will be duplicated in the annealing stage, significantly affecting the DNA level produced during amplification. Dry storage condition has a relatively low success rate compared to humid condition for STR analysis process. This difference was seen in the results of DNA touch levels (table 1), where the highest DNA level occurred at the lowest mean temperature and highest humidity (Group I) and then decreased due to an increase in temperature and humidity on the 7th day, and then the DNA level was increased again on the 14th day following a decrease in temperature and an increase in humidity.

The increase in temperature was related to UV radiation in sunlight which causes DNA damage. Other supporting factors are pressure and the type of surface where the DNA touch was attached. The pressure produced by the face shield strap was influenced by the elasticity of the strap, which was correlated to the respondent's head size. Pressure could increase DNA transfer on the skin surface, which affects the amount of DNA stored. Sponge, used as the face shield surface, is a porous surface and might also affect the amount of DNA due to partial migration into the substrate.
CONCLUSION

The conclusion from the results of the analysis in this study is that there is an effect of time exposure in the time range (1, 7, and 14 days) with environmental temperature on the quality of DNA touch on the face shield using STR CODIS – TH01 and D18S51. Detection of the visualization of the CODIS – TH01 and D18S51 STR PCR results showed bands on gel electrophoresis on days 1, 7, and 14.

AUTHORS’ CONTRIBUTIONS
All authors contributed equally to this work.

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DATA AVAILABILITY STATEMENT
The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT
The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data results from the author’s research and has never been published in other journals.

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