Problem
At the Institute for Forensic Medicine of Munich University, approximately 160 physical examinations in cases of suspected sexual violence are carried out yearly. 99% of these cases involve women. The number of cases in which condom use plays a roll has doubled since 1994. The increasing number of sexual assault cases where the use of condoms were reported lead to the necessity of condom residue analysis. If one wants to give evidence of condom residues, one must first of all know what substances to expect on condom surfaces. The coating of lubricated condoms can be subdivided into two components: microscopically provable particles and chemically analyzable substances. In the case of microscopically provable particles, cornstarch granules are predominant. The granules simplify the unrolling of the condoms during the manufacturing process. For the same reason, polyethylene powder is additionally added to about 80% of the condoms. Until recently, a lycopodium spore powder was applied instead of polyethylene. Primary among the chemically provable substances composing lubricants are silicones. On some condoms the spermicide nonoxynol-9 is additionally applied.

Material
Unused condoms:
54 condom brands available in Germany were examined.

Condom use:
6 volunteer couples performed repeated vaginal intercourse using condoms. Immediately following the first incident a vaginal swab was prepared. After further intercourse, swabs
were taken on the first or second, third and fourth or fifth day following coitus in order to examine them for condom residues. All swabs were then smeared onto slices.

Methods

Light Microscopy:
Swabs were taken from the surface of unused condoms. Smears were produced from these and from vaginal swabs, which, after specification and HE-Coloring, were examined for corn starch granules, polyethylene particles and lycopodium spores.

GC/MS:
The swabs from unused condoms as well as the vaginal swabs were first washed in 5ml TRIS Puffer (pH 7.4). A basic extraction followed (Table 1).

Table 1: Steps in basic extraction.

<table>
<thead>
<tr>
<th></th>
<th>Basic extraction</th>
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<tbody>
<tr>
<td>1.</td>
<td>1 ml washing solution</td>
</tr>
<tr>
<td></td>
<td>+ 1 ml ammonium buffer (pH 8.9)</td>
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<tr>
<td></td>
<td>+ 5 ml ether/ethylacetat (1:1)</td>
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<tr>
<td>2.</td>
<td>precipitation of organic layer</td>
</tr>
<tr>
<td>3.</td>
<td>Evaporation (N₂)</td>
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<tr>
<td>4.</td>
<td>Derivatization (BSTFA/1% TCMS)</td>
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<tr>
<td>5.</td>
<td>1 µL injection (GC/MS)</td>
</tr>
</tbody>
</table>

The GC method was conducted under following conditions: GC Agilent 5890; injector temperature 270°C; interface temperature 300°C; gradient 100°C, isotherm 2min, rate
20°C/min, 300°C, isotherm 5 min; GC-column Varian VF-5MS, 25 mx0.25 mmx 0.25 µm; 1 µL injection volume, split less. MS conditions were: Agilent MSD 5972; full scan mode, m/z 50-550.

Results

Light microscopy:

Unused condoms:

The results of qualitative proof of said particles are gathered in the following graph (Table 2).

Table 2: Microscopic proof of particles from the surface on unused condoms (54 varieties)

<table>
<thead>
<tr>
<th>corn starch granules</th>
<th>polyethylen particles</th>
<th>lycopodium spores</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>64,8</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>27,8</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>5,5</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1,9</td>
</tr>
</tbody>
</table>

Vaginal swabs after condom use:

In the vaginal swabs taken from the volunteer couples, considerable starch granules could be detected, especially one day later. In two out of six cases, starch granules could be found even four day post coitus. In one case, lycopodium spores could be found only within 24 hours following intercourse. In just one case could they be traced up to four days post coitus. At any rate, polyethylene has not yet been detected in genital swabs. There are many reasons for this. Polyethylene is fundamentally not colorable, not even using histological staining methods. Recognizing uncolored particles, which are often smaller than corn starch granules, is difficult
with a colored swab. Moreover, polyethylene particles are added in limited concentrations as corn starch granules to coatings. Polyethylene is also not fixable with formalin or alcohol so that the particles can be rinsed away in the additional coloring process of the slide preparation and finally are no longer contained in the smear.

GC/MS:

Unused condoms:

Chromatograms of all 54 condom surfaces possess a shared similarity. Even the chromatogram from dry and lubricated condoms barely differ. The mass spectra from 5 to 11 peaks of each chromatogram were ascertained and filed in a data bank. Only individual condom brands indicated conspicuous differences. The GC-analyses of vaginal swabs without provided intercourse and condom use showed, in the rule, more divergent characteristics.

Vaginal swabs after condom use:

Figure 1 shows the comparison of a vaginal swab following condom use with that taken from an unused condom of the same brand. The spectra comparison was made possible thanks to the data bank. Proof based on spectra has so far only been attainable from swabs prepared no later than one day after sexual intercourse.
Discussion

The examination results of condom surfaces based on microscopy and the GC/MS methods show that all condoms possess superficial characteristics which can be represented in vaginal swabs. Regarding the GC/MS technique, the application of a data bank for condom identification is imperative. A retrieval system calculated the match with 95% accuracy. Microscopically, starch granules are the easiest to prove. Proof of starch granules on vaginal swabs gives evidence that sexual intercourse has taken place using a condom. Proof of the less frequently occurring lycopodium spores can corroborate this evidence. Both particles are
not specific to condoms, since they also appear in medication and cosmetics and on rubber gloves.

The greatest chance of indicating condom use through light microscopy is within one day post coitus and, in exceptional cases, up to four days after intercourse.

With the GC/MS method, obvious condom use can be proven, but only up to one day post coitus.

Conclusion

- Microscopy can, in most cases, only provide a clue to condom use.
- Through the GC/MS method, condom use can be blatantly proved. Nonetheless, other experiments are necessary in order to increase the number of references in the data bank. Experiences must be collected from routine case work with swabs.
- Through the GC/MS method the type of condom can also be ascertained. However, the results established through microscopy must not be underestimated.

References


Blackledge R D, Vincenti M, (1994) Identification of polydimethylsiloxane lubricant traces from latex condoms in cases of sexual assault. JFSS 34, 245-256
