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RNA Profiling: More Help in Forensic Serology

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Abstract

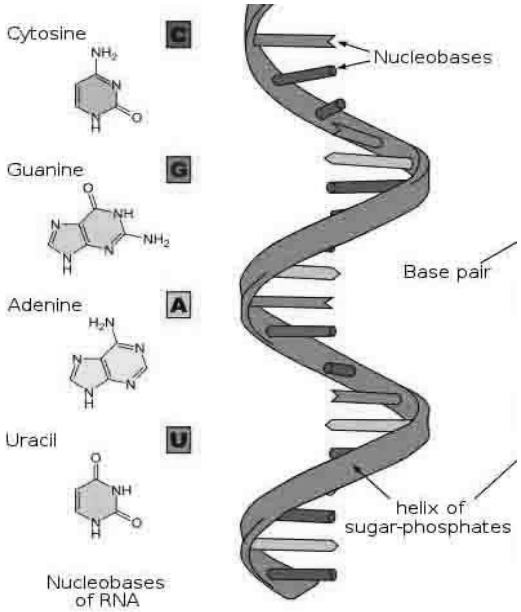
RNA profiling or more specifically messenger RNA (mRNA) profiling is a very useful method to study the body fluids. Various type of body fluids can be found from a crime scene. Because of the changes in the fluids over period of time, it requires more improvise identification method. To study the different biomarkers in different body fluids which is not distinguishable in general process or to analyse the body fluids present in back spatter of a gun, RNA typing or profiling is a safe process. The difference between menstrual blood and peripheral blood, nasal mucosa and vaginal mucosa can be very important in a case. Most of cases DNA profiling for those body fluids are sensitive than RNA profiling. Results of those process show false positive results more which affect the case progress. RNA profiling helps to establish the correlation between DNA profiling in those cases. Addition of many pre mRNAs form a circular RNA (cRNA) which plays the most important role in RNA profiling.

Keywords: Difference between Body Fluids; RNA Profiling; More Accurate Results; Microrna; Forensic Serology.

Introduction

RNA or ribonucleic acid, a single chain polyribonucleo -tide controlled every important function in the early life this fact is not very acceptable. More clearly RNA was first genetic material. It is also not much clear how it helped as first biocatalyst1. Though it is not so sure still there are some enzymes – ribozymes are made up of RNA. Through some medical changes it formed into DNA. Now RNA acts as carrier of coded genetic information from DNA to cytoplasm to help in protein and enzyme synthesis.

RNA is single stranded polynucleotides but some places it shows partially double stranded due to folding or coiling of the single strand. There are 70-12000 ribonucleotides joined from start to end2. The backbone of RNA is made up of alternate residue of phosphate and ribose sugar. These phosphate combines with 5′ of its sugar and 3′ of next sugar and form a DNA like structure. There are four types of nitrogen bases present in RNA 1) adenine 2) guanine 3) cytosine 4) uracil. Nitrogen bases arrange as a complementary to respective DNA template. There are 3 types of RNA mainly help in every major classes. It also acts as a genetic material in some viruses.



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Collected body fluids from the crime scene are major help to solve a particular crime. RNA was first mentioned in forensic literature by oehmichem et al in 1984 reporting on post mortal biosynthesis of DNA and RNA[3]. We can find RNA from dried blood stain, saliva mucosa, vaginal mucosa, buccal mucosa, semen. If there is very little amount of evidence, we still can apply PCR (polymerase chain reaction) to increase the amount of that specific biological fluid. To work with body fluids, we have to be cautious about degradation of its. We should consider sensitive and stable bio markers to prevent the effect of degradation, for that we can consider microRNAs which are less prone to degradation due to their small size and stable structure.[4] RNA profiling also helps to investigate postmortem time interval, age of the wound, identify the exact firearms used for murder from numerous numbers of fire arms in a gang fighting or robbery. But to get exact desirable results we should follow some precautions-1) how unbiasly we can execute the identification 2) distinguishable process adapt to extraction of RNA than DNA 3) consider physiological. Environmental factors.

Through this article I am giving the idea of more use of RNAs in forensic investigation as it gives more accurate result than DNA profiling for body fluids and reduces false positive results. With the guidance of proper procedures, we can solve more cold cases. We will be able to give those cases a proper breakthrough and it will be easier to narrow down the suspects.

Methods

Sample collection:

We can easily collect set of control samples of body fluids and tissues and through the process of evidence collection. This control set includes blood, salina, vaginal mucosa, menstrual secretion, semen and skin sampling from four individuals[5]. To called fresh nasal mucosa samples we have to consider both nostrils and for variation the more number will be beneficial.by using a single cotton swab per nostril the sample from 22 individuals were taken. From seven of these individuals were suffering from cold. A total of 11 nosebleed samples from six donors were collected on tissue paper[6]. From 10 donors the samples of sweat, tear, and urine has been collected on cotton swabs. We have to maintain the distance to avoid skin contact, semen samples are collected from four individuals. Two samples from fertile individuals and other two from vasectomised individuals. After collection we should keep those swabs were air dried and in room temperature in the dark until we used. The collection of 10 samples were collected and stored at -20c and waste materials from those specimens were also taken. Penile swabs were collected by 20 donors using 4N6FLOQ swabs™ with active drying system (Copan liagnostics)[7]. Before collecting any samples, consent has taken rom informed voluntary donors.

Marker name	Tissue	[primer] μM	Forward primer (5′–3′) Reversed primer (5′–3′)	Size (bp)	Dye	Reference
CD93	Blood	0.25	ACCAGTACAGTCCGACAC TTGCTAAGATTCCAGTCCAG	151	NED™	8
HBB	Blood	0.035	GCACGTGGATCCTGAGAAC ATGGGCCAGCACACAGAC	61	FAM™	8
HTN3	Saliva	0.2	GCAAAGAGACATCATGGGTA GCCAGTCAAACCTCCATAATC	134	VIC™	8
STATH	Saliva/nasal mucosa	0.3	TTTGCCTTCATCTTGGCTCT CCCATAACCGAATCTTCCAA	93	FAM™	8
SEMG1	Seminal fluid	0.8	GGAAGATGACAGTGATCGT CAACTGACACCTTGATATTGG	91	FAM™	8
PRM1	Spermatozoa	0.3	AGACAAAGAAGTCGCAGAC TACATCGCGGTCTGTACC	146	NED™	8
CYP2B7P1	Vaginal mucosa	0.8	AGTCTACCAGGGATATGGCATG CTATCAGACACTGAGCCTCGTCC	141	VIC®	9
MUC4	Vaginal mucosa	0.8	CTGCTACAATCAAGGCCA AAGGGAAGTTCTAGGTTGAC	88	FAM™	8
MMP7	Menstrual secretion	0.8	GAACAGGCTCAGGACTATCTC TTAACATTCCAGTTATAGGTAGGCC	107	VIC®	8
MMP10	Menstrual secretion	0.1	GCATCTTGCATTCCTTGTGCTGTTG GGTATTGCTGGGCAAGATCCTTGTT	76	VIC®	9
MMP11	Menstrual secretion	0.4	CAACCGACAGAAGAGGTTCG GAACCGAAGGATCCTGTAGG	71	NED™	9

Fig. 1: van den Berge & Sijen, 2017.

Presumptive tests

After collecting all the specimen presumptive tests for every specimen are required individually. For semen the rapid stain identification series (RSID) is used and to observe the presence of spermatozoa, microscopic analysis has been also conducted. For saliva sample RSID has been also performed. TB testing for blood was performed by transferring biological material to a with water moistened filter paper, to which on drop of tetra bass solution in 10% acidic acid was added10.one drop of barium per oxide solution was added next10. The colour formation will observe according to criteria.

RNA extraction

To extract RNA from biological sample we can proceed with commercial extraction kits.

1. Mirvana™mi RNA isolation kit.[11]

For RNA isolation we used the protocol described by lindenbergh et al.[12] DNase is used is to degrade. DNA in the process of RNA isolation. The RNA extracts were treated with DNase collected swabs were processed carefully. The swabs from excised from the nosebleed tissues were cut 1cm.[2] If the amount of extracted DNA was below 1ng then RNA extracts from ethanol precipitated was prioritize in the reverse transcription. To separate non sperm fraction and sperm fraction, the use of customized mild lysis is more effective. This mild lysis buffer is made up of phosphate buffered saline (PBS). which is composed with 1.6 mg proteinase k and 10µM ribonucleoside vanadyl complex, these two inhibits various ribonucleases, in 50µM of mild lysis buffer the swabs are incubated for 56 c for 20min. After using a QIA shredder column the lysate is separated from carrier material, during centrifugation at 11,000rpm. The non sperm fraction and sperm fraction got separated. Pellet of sperm fraction is washed using 400µL PBS buffer with 10 µM ribonucleoside vanadyl complex and after that it is centrifuged at 13.200rpm for 5min. Then the mirvana mi RNA is isolation kit is used for binding lysis, then the supernatant of non-sperm fraction after centrifugation is treated with lysis buffer and then addition of homogenate additive is placed.

RNA analysis

RNA analysis comprised of CDNA synthesis 19-plex reverse transcriptase PCR product purification and analyse using gene mapper. RNA marker signals help in identification of body fluids. There are several markers for each body fluids. Markers for blood (HBB, CD93, AMICA1) markers for saliva (STATH, HTN3, KRT13) and for mucosa, SPRR2A is used is general. Markers for semen (PRM1 and SEMG1) and for fertile men PRM1 is used. MUC4 for specific vaginal mucosa markers and some markers for menstrual secretion. Different types of marker help to differentiate between different body fluids. The marker for nasal mucosa did not show cross reaction with peripheral, BPIFA1 markers which can be used to differential between peripheral blood and nasal blood. Certain markers don't show the positive results for other body fluids.it discriminates between different type of body fluids.

Result

Through the process of RNA typing, it helps us to differentiate between body fluids. But in case of DNA profiling, it provides the accurate results of the source of the individuals. RNA profiling or typing concludes the cell types present in evidentiary body fluids. The process of RNA typing is followed by clear guidelines. In case of vaginal mucosa due to presence of microbes and bacteria DNA profiling shows false positive result. But in case of examination of skin cells DNA profiling shows more false positive results for whenever skin contact with body fluids, though for detailed cell type information RNA typing is more helpful.

Conclusion

RNA profiling is very recognized method for body fluid identification though it is used as a supplementary method. We can identify large number of body fluids through this process. It helps us to detect the type of body fluids through the RNA marker from a very trace amount. In some of the cases where DNA profiling is responsible as false positive results, there RNA typing comes as a blessing. It is helpful for very degraded body or evidence. In order to obtain accurate results of this method for RNA typing we require to maintain the chain of custody and also unbiased forensic interpretation. But the use of mRNA markers can cause of cross reaction. To identify a human body fluid accurately, we need to consider those cross reaction.

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