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Title: Storing and detecting nucleic acid administered to a solid carrier

The invention relates to the field of medicine. More particularly the invention relates to diagnosis. The invention especially relates to detection and/or quantification of a nucleic acid of interest in a sample.

Infections with pathogens are commonly observed all over the world. Such infections for instance include viral, bacterial, fungal and parasite infections. Early diagnosis of an infection is often preferred for efficient treatment, which can prevent severe pathological symptoms. Sometimes, early diagnosis is a prerequisite for a possibility of treatment, for extending a life-time and/or for improving the quality of life. Examples of such infections comprise Hepatitis C virus, Hepatitis B virus, tuberculosis, malaria and HIV, such as HIV-1.

The HIV-1 epidemic spreads readily over the world and with the spread of the virus the circulating subtypes of the HIV-1 virus are no longer restricted to one geographical place on earth. This development requires an application of (nucleic acid) diagnostic tests that can detect all subtypes of the HIV virus with equal accuracy and precision.

The globalization of the epidemic and especially the severity in resource-poor countries (sub-Saharan Africa and south-east Asia) has driven the developed world to assist the fighting of infection in the less-developed countries with access programs to pharmaceuticals. However, in order to efficiently help HIV-1 infected people in the less-developed world there has to be suitable diagnostics in place, for instance to monitor the efficacy of a treatment. Such suitable diagnostics are especially viral load assays that measure the amount of HIV-1 RNA in a bodily fluid, such as blood, blood plasma, mothers milk, semen, lymph fluid, sputum, liquor, saliva and/or urine of infected individuals. Likewise, suitable nucleic acid load assays are desired for other infection-related diseases.

The current status of suitable nucleic acid load assays is of such a high technical standard that these are not easily transferred to areas located at considerable distance of technological resources such as in sub-Saharan Africa and/or south-east Asia. This requires significant investment in infrastructure that is impossible in the setting of these regions. An alternative solution is an analysis of patient samples in laboratories in the developed world (for instance Europe and north America). This alternative is however impossible to exercise due to logistic problems. Body fluid samples such as blood or blood plasma samples of individuals have to be shipped to the laboratory in frozen conditions at temperatures well below 0°C, usually in a box with dry ice. This way of sending clinical material is very expensive and requires the availability of dry ice at the place of shipment. The latter is often not the case in countries of the less-developed world. Because of the costs and the unavailability of dry ice the remote testing of samples in laboratories ("service testing") is not an option for these countries and for infected people in these countries. The pharmaceutical access programs for less-developed countries can only be successful if diagnosis is optimized.

The invention provides a method for detecting a nucleic acid of interest in at least one sample, comprising:

- administering said sample to a solid carrier capable of at least in part absorbing said sample;
- drying said carrier;
- providing at least a representative part of said carrier to a nucleic acid isolation solution so that a representative amount of said nucleic acid is extracted from said carrier; and
- detecting said representative amount of said nucleic acid.

With a method of the invention a sample, such as a body fluid sample, is stabilized in such a way that it can be shipped from the site of taking (for instance local hospital or lab in a less-developed country) and be sent to a

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service testing laboratory elsewhere in the world by normal logistics means, for instance normal postal service. Said method makes possible shipment of a dried sample via surface mail to a remote lab for detection of a nucleic acid of interest present in a sample.

A method of the invention can be performed with techniques known in the art. For instance, a defined amount of liquid sample can be administered to a solid carrier using a pipette. Said solid carrier is not critical and can comprise any solid carrier known in the art, as long as it is capable of at least in part absorbing said sample. For instance, said solid sample can comprise silica. Preferably, however, said solid carrier comprises filter paper. A filter paper is well capable of absorbing a liquid sample, while it is of light weight. This is very important for postal services. Said filter paper can comprise plain untreated filter paper like the 903 paper from Schleicher and Schuell, or treated filter paper that immobilizes nucleic acid for rapid purification. The two examples in the field are IsoCode paper from Schleicher and Schuell or FTA-treated paper from Whatman, which both are bactericidal, fungicidal and virucidal, inhibits the growth of bacteria and fungi and kills viruses that come in contact with the matrix, allowing for safe sample handling and sample stability. Another example of a suitable carrier to store and transport dried fluid samples is a device designed by Lifestock (US 5139742) This device contains a small knife with a capillary tube directly attached to it, which enables collection, storage and transportation of blood that dries in the capillary tube thereby comparable to the method with the filter paper.

Said carrier can be dried by different techniques known in the art. Preferably said sample is dried to the air. This is inexpensive and effective.

By a representative part of a carrier is meant a part which is indicative for the amount of sample administered to said solid carrier. For instance said representative part can comprise the whole of said sample. Said representative part can also comprise half the amount of said sample. In that case the other half can be used to perform a second experiment. Said second experiment can

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be a different experiment, or can be a similar experiment. In the latter case a certain result can be obtained *in duplo*, which is more accurate.

By a representative amount of nucleic acid is meant an amount which is indicative for the amount of nucleic acid present in said sample. Said representative amount can comprise the whole amount of said nucleic acid present in said sample. Alternatively, said representative amount can comprise a part of said nucleic acid present in said sample.

Preferably a method of the invention is provided wherein at least 100 µl of sample is administered to said carrier. More preferably, at least 250 µl of sample is administered to said carrier. Most preferably at least 500 µl of sample is administered to said carrier. With a high sample volume, low titers of nucleic acid of interest can still be detected. A major drawback in current detection methods is that only nucleic acid with a concentration above a certain threshold value can be detected. This means that infected individuals with a low load of pathogenic nucleic acid are not diagnosed as being infected and, hence, do not get treatment at an appropriate early timepoint. Until the present invention high amounts of body fluid samples, such as blood or plasma samples, were not suitable for testing because inhibitory effects were observed. For instance, it has been reported that hemoglobin and carbonic anhydrase present in whole blood interfere with the polymerase chain reaction (4). PCR mixtures became deep brown because of the elution of heme and its degradation products from filter blotters (5). To improve a PCR amplification, specimens were treated with methanol before the PCR reaction (6). This involves an extra step, with risk of contaminations and less reliable test results. Besides, chelating metal ions are reported to act as catalysts for the breakdown of DNA at a high temperature with low ionic strength (7).

Surprisingly, with a method of the invention high amounts of sample can be stored on a dried solid carrier and subsequently analysed. None of the above-mentioned inhibitory effects are observed and treatment with methanol as a precautionary measure is not necessary. With a method of the invention it

is now possible to analyse large samples for the presence of a nucleic acid of interest. Hence, low concentrations of a nucleic acid of interest in a sample can now be detected. A method of the invention is suitable for screening individuals for the presence of one or more specific pathogens, such as HIV-1. Alternatively an individual suffering from a disease, or at risk of suffering from a disease, can be investigated with a method of the invention. Once one or more foreign nucleic acid(s) are found, it can be determined to which kind of microorganism it belongs. This can for instance be done by hybridisation protocols using different kinds of probes. Alternatively, techniques to determine the sequence of the nucleic acid and analyzing this sequence for homology with any known sequences can be performed. In one embodiment the invention therefore provides a method of the invention, comprising identifying said nucleic acid of interest.

In yet another embodiment a method of the invention is provided wherein said nucleic acid is quantified. Surprisingly it is not only possible with a method of the invention to detect a nucleic acid of interest, but also to determine the amount of said nucleic acid present in said sample. With a method of the invention no significant amount of nucleic acid is lost/broken down during storage and/or during the isolation and/or detection procedure. In the example is shown that, if a method of the invention is used, a measured amount of a nucleic acid of interest from a dried sample stored on a solid carrier is comparable to the measured amount of said nucleic acid of interest when said sample is directly subjected to analysis.

Dried samples on a solid carrier can now be investigated for the amount of pathogenic nucleic acid present. Hence, not only the presence, but also the stage of a disease can now be determined. It is now possible to diagnose individuals of regions with insufficient facilities, such as inhabitants from resource-poor countries. A body fluid sample, such as a blood sample, can be collected on a solid carrier such as a filter paper. Said filter paper can be sent

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to a laboratory, for instance in Western Europe. Subsequently the amount of a nucleic acid of interest can be determined and, hence, the status of a disease. It can also be determined whether a treatment is effective by determining whether the amount of said nucleic acid declines over time.

In one aspect the invention provides a method of the invention wherein said solid carrier is provided with at least two samples. Preferably, said samples are obtained from the same individual. More preferably, said samples are obtained from the same body fluid of said individual. Said samples can for instance comprise two aliquots of blood. Each of said blood samples can be tested independently by a method of the invention. This way the presence and/or the amount of a nucleic acid of interest in the blood of said individual can be tested *in duplo*. This results in more accurate results. Moreover, each sample can be tested by different persons/different institutes. Errors made by individuals and/or errors because of unreliable equipment can be revealed by an independent second measurement.

In an alternative embodiment, said two samples are used for different purposes. For instance, one sample can be tested for the amount of viral nucleic acid, while the other sample can be tested for the amount of bacterial nucleic acid. Both samples can be used to obtain ratios between viral nucleic acid and chromosomal DNA (as is important for CMV), or for ratio between mitochondrial vs cellular DNA/RNA or to determine ratios involving mRNA's or rebosomal RNA's. Many of these tests, however can also be carried out using only one sample.

In one embodiment, said solid carrier comprises a series of at least two samples, taken at different time points. This way a course of a disease can be followed over time. This embodiment is also particularly suitable for determining whether a treatment is effective. For instance, a sample can be administered to said solid carrier at the start of a treatment, and at regular intervals afterwards. In this way the said solid carrier does not only serve the

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purpose of transportation device, but also for stable storage at ambient temperatures without degradation of the nucleic acid of interest. Such solid carrier can be sent to a suitable institute after a certain amount of time. It can then be established whether a treatment is efficient by quantifying the amount of pathogenic nucleic acid in each sample by a method of the invention. It can be established whether said amount declines over time.

Likewise, samples can be taken from a diseased individual at regular intervals. The amount of pathogenic nucleic acid in each sample can subsequently be quantified. This provides more insight into the course of said disease; for instance whether the amount of pathogenic nucleic acid increases over time, etc.

It is not necessary to send each sample separately to said institute. A number of them can be collected over time and be sent at once. This saves time and money. Moreover, because said samples are sent together, no storing and sorting of separately sent samples is necessary. A risk of samples getting lost is decreased.

To even more accurately quantify an amount of a nucleic acid of interest with a method of the invention, a known amount of a reference nucleic acid can be administered to said solid carrier. Said reference nucleic acid can be quantified, as well as a nucleic acid of interest. The accuracy of a quantification of a nucleic acid of interest can be determined by comparing a measured amount of said reference nucleic acid with the administered amount of said reference nucleic acid. If said measured amount differs slightly from the administered amount, the same is likely to be true for said nucleic acid of interest. Hence, a measured amount of said nucleic acid of interest can be corrected to obtain an even more accurate result.

Moreover, with a reference nucleic acid a representative part comprising a part of a sample can be determined. If a representative part comprising a part of said sample is provided to said nucleic acid isolation buffer, and the

measured amount of reference nucleic acid appears to be one third of the administered amount, it indicates that the measured amount of a nucleic acid of interest is also approximately one third of the amount present in said sample. Hence, a reference nucleic acid shows with which factor a measured amount of nucleic acid of the invention should be multiplied if a part of said sample is provided to said nucleic acid isolation buffer. A reference nucleic acid is preferred which spreads along said solid carrier essentially the same way as said nucleic acid of interest. In that case it makes no significant difference which part of said solid carrier is used in a method of the invention.

Another way of accurately quanification of the nucleic acid of interest is by relating it to the amount of other nucleic acids. In this way it is possible, in either the same reaction or in separate reactions, in either the same sample or one of the other samples, to establish a ratio between any DNA and DNA, any RNA and DNA or vice-versa, and any RNA and RNA target. The invention could comprise applications to determine the ratio between nucleic acids from the host, e.g. the amount of mitochondrial DNA versus nuclear DNA as a measure of response to certain HIV therapy, or between nucleic acids from a pathogen versus those from the host, e.g. the amount of CMV DNA versus host nuclear DNA. Other applications could comprise the amount of mRNA per cell within the field of gene expression profiling.

The invention also provides a method of the invention wherein said representative part comprises essentially the whole of said at least one sample. It is shown by the present inventors that even with a large sample a reliable detection and/or quantification of a nucleic acid of interest is possible with a method of the invention. If the whole of a large sample is used, even low concentrations of nucleic acid can be detected and quantified.

A representative part of said carrier can be provided by cutting a visible spot out of a solid carrier such as filter paper. This cutting can be done using a normal pair of scissors, but can also be completely automated, e.g. using

equipment from Wallack to punch out equal surfaces of the solid carrier. Another way of easying this type of punching by hand, would be to pre-punch said solid carrier, after which the sample is applied and dried. At arrival in the laboratory for analysis, this pre-punched part can be easy punched out completely by either specially designed devices or existing devices as a Safe-lock tube from Eppendorf. Also a representative part of said spot can be used. However, it is preferred to use the whole carrier, as it is then ensured that the whole sample is measured. According to the present invention, a solid carrier such as filter paper does not significantly influence a detection and/or quantification of a nucleic acid of interest. Therefore, a method of the invention is preferred wherein said representative part comprises essentially the whole of said solid carrier.

If in a method of the invention said solid carrier is provided with at least two samples, said representative part preferably comprises one of said samples. Said representative part can be used for detecting and/or quantifying a nucleic acid of interest. As has been explained above, a representative part comprising said second sample can be used for a measurement *in duplo*. Alternatively, an other measurement can be performed.

In one aspect a method of the invention is provided wherein said nucleic acid isolation solution comprises a chaotropic nucleic acid isolation lysis buffer. More preferably, a nucleic acid isolation buffer as described by Boom et al is used. Preferably said solid carrier comprises filter paper, since filter paper is cheap, well capable of absorbing a liquid sample and of light weight which facilitates transport. Typically, elution of the said nucleic acid takes at least 30 minutes at room-temperature or even shorter at elevated temperatures, whereas nucleic acid from a bodily fluid that is applied direcly to the lysis buffer is typically released within 10 minutes. Elevated temperatures will facilitate efficient and quick elution of the nucleic acid from the solid carrier.

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A method of the invention is particularly suitable for detecting viral nucleic acid, especially retroviral nucleic acid. Viral nucleic acid can be present in a latent stage, which can last for a considerable time. Moreover, a virus such as HIV, HTLV and HHV is often present during a considerable time within an individual before said individual experiences any significant symptoms. During that time said virus is often already transmittable to other persons. Moreover, treatment in an early stage can improve a chance of recovery, prolong a life-time and/or improve the quality of life. Therefore it is particularly important to check an individual for the presence of viral nucleic acid with a method of the invention. Viral nucleic acids to be detected include sequences from Hepatitis A, B, C, parvovirus, etc. Preferably, said viral nucleic acid comprises HIV and/or HTLV. More preferably, said viral nucleic acid comprises HIV-1.

In one embodiment a method of the invention is provided, wherein said method comprises genotyping a mutant. This is especially useful for organisms with a fast-changing genome, such as (retro)viruses. Genotyping is for instance useful for determining whether a certain treatment is likely to be suitable for an individual patient. Moreover, if a new mutant is found, an existing pharmaceutical preparation can be adapted, or a new medicament can be developed.

A method of the invention is particularly suitable for detecting a nucleic acid of interest in a body fluid, such as blood, plasma, mothers milk, semen, lymph fluid, serum, sputum, liquor, saliva, and/or urine. A sample of such body fluid is easy to obtain. Obtaining such sample does not cause much inconvenience to an individual, which would be the case if for instance a biopsy were taken. Moreover, obtaining a body fluid sample does not require special equipment, which is often lacking in less developed countries and in remote areas.

Preferably a method of the invention is provided wherein said sample comprises a droplet of whole blood from a finger or heel puncture. Such finger or heel puncture is commonly taken from newborns, so that said material is often available without further bothering the individuals. In another preferred embodiment said sample is a plasma sample. A plasma sample allows more accurate measurement of an amount of free virus particles, whereas for instance a blood sample also includes viral nucleic acid integrated within cells. Measurement of free virus particles, essentially without viral nucleic acid integrated within cells, is particularly indicative for characteristics such as viral virulence, viral spread, etcetera. Hence, a plasma sample is especially preferred if such characteristics are to be determined.

To make sure that at least the minimally necessary amount of bodily fluids is collected, a pre-determined surface can be printed or applied in any other way to the solid carrier. The meaning is that the surface will be completely filled with the requested bodily fluid. Since an equal volume of whole blood will result in a smaller covered surface than a similar amount of bodily fluids, e.g. serum, or mother milk, it might be necessary to have several sufaces printed for various bodily fluids on the same solid carrier, or to have carriers designed specifically for certain bodily fluids. In this way it is ensured that minimally the correct amount of bodily fluid is collected.

For detection of a nucleic acid of interest often an amplification step (such as PCR or NASBA) is necessary. Amplified nucleic acid can subsequently be detected using known methods in the art. A method of the invention therefore preferably comprises an amplification step. Preferably said amplification comprises real-time monitored amplification. Produced nucleic acid is directly made visible during such amplification reaction. This can for instance be achieved by molecular beacon probes or other types of probes. Once these probes anneal to a template, a fluorescence signal can be generated which can be monitored during an amplification reaction. The intensity of

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fluorescence is indicative for the amount of nucleic acid generated. A calibration curve can be created using known amounts of nucleic acid. A fluorescence signal from a sample with an unknown amount of nucleic acid can then be compared with said calibration curve. This way the amount of said nucleic acid in said sample can be determined, because the intensity of fluorescence and the amount of nucleic acid are correlated.

In another embodiment said nucleic acid detection and/or quantification is performed with an end-point read-out system. Such systems for instance comprises a colorimetric detection, an enzymatic assay, and/or a dipstick.

The invention also provides a use of a dried solid carrier provided with a sample for detecting, identifying and/or quantifying a nucleic acid of interest in said sample. As has been described previously, said dried solid carrier can be stored and transported easily, after which reliable nucleic acid detection and quantification can be carried out. Preferably said solid carrier comprises at least the equivalent of 100 µl of blood or a derivative thereof in dried form. Said carrier comprises more preferably at least 250 µl, most preferably at least 500 µl of blood or a derivative thereof in a dried form. With a use of the invention a large volume of sample can be investigated, allowing detection and/or quantification of a low concentration of nucleic acid. With a derivative of blood is meant at least part of a component of blood, such as serum and/or plasma. Blood which has been modified artificially is also within the scope of a derivative of blood. The way the solid carrier is designed, it could well contain the carrier itself that can absorb the bodily fluid, linked to a part of paper or surface on which information can be written or printed, e.g. information about the patient, date of sampling or more dates of sampling, therapy regimen, barcodes, ID-numbers, etc. Such a surface specifically for this type of information is unequivocally linked t the carrier with sample, thereby making sure the information is not lost. Typically this type of surfaces can contain

much more information than a tube can hold. Logically, any variation on this theme is possible.

The invention also comprises a kit of parts for detecting, identifying and/or quantifying a nucleic acid of interest in a sample, comprising:
- a solid carrier capable of at least in part absorbing said sample; and
- a nucleic acid isolation solution.

Preferably said kit further comprises means for nucleic acid amplification. Said means for instance comprise means for real-time monitored amplification, and/or means for amplification with end-point detection/quantification. A kit of parts of the invention is for instance useful in resource-poor countries with a few hospitals. Such hospitals can distribute said solid carriers, such as filter papers, among inhabitants in remote areas. Once samples have been collected, they can be stored and transported to such hospitals. If said hospital is properly equipped, said samples can be investigated using said nucleic acid isolation solution. Of course, also hospitals in developed countries can use a kit of part of the invention for collecting and testing a sample. A kit of parts for detection, identification and/or quantification could well contain a collection of materials necessary to safely draw the bodily fluid from the patient. Logically with external bodily fluids like urine, mothers milk or saliva, other safety precautions will have to be taken than when internal bodily fluids are samples like blood, plasma, serum, or lymph drain. For the internal bodily fluids, one can compose a kit that contains a solid carrier capable of at least in part absorbing said sample, and a nucleic acid isolation solution, next to a pair of examination gloves, a alcohol swab to clean the skin, a finger or heel puncture device, a bandage, an envelope e.g. with the address of the destinated laboratory as well as with a space for an identification number or patient code, and coated inside for safe postal transportation, and a desiccator to keep the sample dry and to prevent it from fungal or bacterial growth. Other possibilities for the collection device

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could comprise of specially designed devices for one-time use, like a device described in patent number US5139742: Disposable liquid testing device by Livestock Control Holding B.V. in Amersfoort, the Netherlands. Any combination of these items, or replaced for other type of items/devices to be used for storage and/or transportation of any nucleic acid containing bodily fluids is possible.

A solid carrier comprising at least the equivalent of 500 µl of blood or a derivative thereof in dried form is also herewith provided. Preferably, said solid carrier comprises at least two samples. If said samples are of the same kind, said samples can both be tested separately, resulting in an *in duplo* test. Furthermore, a result of a first test can be controlled by independently testing the other sample. Alternatively, said samples are used for different purposes.

A solid carrier of the invention comprising a series of samples obtained at different data is also herewith provided. As has been described before, such solid carrier is suitable for following a course of a disease over time, and/or for testing whether a certain treatment is effective. Preferably a solid carrier of the invention comprises a known amount of a reference nucleic acid.

The invention is further explained in the following example. The example only serves to clarify the invention. It does not limit the scope of the invention in any way. Alternative embodiments are also within the scope of the present invention.

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Examples

Example 1

Blood and blood plasma were spotted in 50 µl droplets on S&S 903 paper (Schleicher & Schull) and dried in the air. Simultaneously, 200 µl of the same blood and plasma samples were directly added to the lysis buffer as described by Boom et al. (1990). After drying, the spots on the filter paper were kept at ambient temperature for up to 3 weeks and can probably be kept at ambient temperature for months. The spots on the filter paper were excised with a normal pair of scissors and administered to a tube containing lysis buffer as described by Boom et al. (1990). The filter spots of 50 µl blood or plasma were added to three different tubes: 1) a 50 ml tube containing 9 ml lysis buffer, 2) a 15 ml tube containing 15 ml lysis buffer or 3) a 1.5 ml eppendorf tube containing 1 ml lysis buffer.

The tubes were mildly shaken on a shaking platform for 3 hours at ambient temperature. During this incubation the blood or plasma spot dissolves from the filter paper into the lysis buffer. Subsequently the filters were removed from the tubes with a cleaned pair of tweezers. Between tubes the tweezers were subsequently cleaned with hot water-chlorine-hot water-70% alcohol.

To the tubes with lysis buffer and the samples a 1.10^6 copies of a system control RNA molecule were added to allow identification of false negative reactions at a later stage. The system control RNA is amplified with the same primers as the wild-type HIV-1 and detected with a distinguishable probe in the reaction. Due to a length difference the system control RNA can only be amplified and detected in the absence (or very low amounts) of the wild-type HIV-1 RNA.

The nucleic acid now present in the lysis buffer was further purified with the method described by Boom et al (1990) or with dedicated isolation kits

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purchased from Qiagen (Qiagen GmbH, Max Volmer Strasse 4, 40724 Hilden, Germany) or Biomerieux (formerly Organon Teknika, Boseind 15, 5281 RM Boxtel, The Netherlands) and used according to the manufacturer's protocols. The isolated nucleic acid was stored at -80°C until further analysis. Usually 5 µl was used as input in NASBA amplification reactions determining the amount of HIV-1 RNA as described by De Baar et al. (1, 2)

Standard NASBA nucleic acid amplification reactions were performed in a 20µl reaction volume and contained: 40mM Tris-pH 8.5, 70mM KCl, 12mM MgCl2, 5mM dithiotreitol, 1mM dNTP's (each), 2mM rNTP's (each), 0.2µM primer (each), (P1: AAT TCT AAT ACG ACT CAC TAT AGG GAG AGG GGC GCC ACT GCT AGA GA and P2: CTC AAT AAA GCT TGC CTT GA), 0.05µM molecular beacon for the wild-type HIV-1 sequence (MB045: FAM-CGA CGT AGT AGT GTG TGC CCG TCT GTA CGT CG-dabcyl), 0.05µM molecular beacon for the system control RNA (MB054: ROX-CCG ACT CTC TAC ACA CCA GAC AAA AAA CGA GTC GG-dabcyl)

0.05µM molecular beacon for the wild-type HIV-1 sequence, 0.05µM molecular beacon for the system control RNA, 375mM sorbitol, 0.105 µg/µl bovine serum albumin, 6.4 units AMV RT, 32 units T7 RNA polymerase, 0.08 units RNase H and input nucleic acid. The complete mixture, except the enzymes was, prior to adding the enzymes, heated to 65°C in order to denature any secondary structure in the RNA and to allow the primers to anneal. After cooling the mixture to 41°C the enzymes were added. The amplification took place at 41°C for 90 min in a thermostated fluorimeter (CytoFluor 2000 or EasyQ Reader) and the fluorescent signal of the molecular beacon probe was measured every minute.

To achieve quantification, a dilution series of target sequence for a particular primer set was amplified and the time points at which the reactions became positive (the time to positivity, TTP) were plotted against the input amounts of nucleic acid. This way a calibration curve was created that could be used to

read TTP values of reactions with unknown amounts of input and deduce the input amount.

The results of the determinations in example 1 are shown in table1 below. By the results of the system control RNA is appeared that there were no false negative results and all negative data reported in table 1 are true negative data resulting from the absence of HIV-1 sequence or presence at concentrations below the detection limit of the tests.

Patient #	200 µl plasma direct to lysis buffer [A]	200 µl blood direct to lysis buffer [B]	50 µl plasma spotted [B]	200 µl plasma spotted [A]	50 µl blood spotted [B]	200 µl blood spotted [A]
R02-05195	Neg.	Pos.	Neg.	Neg.	Neg.	LQL
R02-05260	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
R02-05179	3.84	Pos.	Pos.	3.86	Pos.	3.86
R02-05183	LQL.	Pos.	Pos.	3.74	Pos.	LQL
R02-05240	3.79	Pos.	Pos.	3.86	Pos.	3.77
R02-05244	3.79	Pos.	Pos.	3.59	Pos.	LQL
R02-05265	3.20	Pos.	Neg.	LQL	Pos.	LQL
R02-05175	5.18	Pos.	Pos.	4.79	Pos.	4.76

A. The results were determined quantitatively as described in the text with TTP measurements. The results are given as the Log number. Neg. indicates a negative result, LQL indicates a positive result, but too low for accurate quantification

B. The determinations of 200 µl blood direct to lysis buffer, 50 µl plasma spotted and 50 µl blood spotted were not performed quantitatively, only qualitative with either a positive (Pos.) or negative (Neg.) result.

The data in table 1 clearly indicate a good correlation between the results obtained with the direct admission of sample to the lysis buffer compared to

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first spotting of the sample on paper, drying and thereafter admission to the lysis buffer.

Example 2

Mother milk of 1 woman, spiked with virus from 6 different isolates in 4 concentrations was spotted in 4 times 50 µl droplets on S&S 903 paper (Schleicher & Schuell), dried on the air and stored for a minimum of one week at ambient temperature. After drying, the spots on the filter paper were kept at ambient temperature for up to 3 weeks and can probably be kept at ambient temperature for months. Simultaneously, 200 µl of the same mother milk samples was directly added to the lysis buffer as described by Boom et al. (1990). The spots on the filter paper were excised with a normal pair of scissors and administered to a tube containing 4 ml lysis buffer as described by Boom et al. (1990).

The tubes were mildly shaken on a shaking platform overnight at ambient temperature. During this incubation the dried spot dissolves from the filter paper into the lysis buffer. Subsequently the filters were removed from the tubes with a cleaned pair of tweezers. Between tubes the tweezers were subsequently cleaned with chlorine-hot water-70% alcohol.

To the tubes with lysis buffer and sample 1.000.000 copies of a system control RNA molecule was added to allow identification of false negative reactions at a later stage. The system control RNA is amplified with the same primers as the wild-type HIV-1 and detected with a distinguishable probe in the reaction. Due to a length difference the system control RNA can only be amplified and detected in the absence (or very low amounts) of the wild-type HIV-1 RNA. The nucleic acid now present in the lysis buffer was further purified with the method described by Boom et al (1990) or with dedicated isolation kits purchased from Qiagen (Qiagen GmbH, Max Volmer Strasse 4, 40724 Hilden, Germany) or Biomerieux (formerly Organon Teknika, Boseind 15, 5281 RM

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Boxtel, The Netherlands) and used according to the manufacturer's protocols. The isolated nucleic acid was stored at -80°C until further analysis. Usually 5 µl was used as input in NASBA amplification reactions determining the amount of HIV-1 RNA as described by De Baar et al.

Standard NASBA nucleic acid amplification reactions were performed in a 20µl reaction volume and contained: 40mM Tris-pH 8.5, 70mM KCl, 12mM MgCl2, 5mM dithiotreitol, 1mM dNTP's (each), 2mM rNTP's (each), 0.2µM primer (each) (P1: AAT TCT AAT ACG ACT CAC TAT AGG GAG AGG GGC GCC ACT GCT AGA GA and P2: CTC AAT AAA GCT TGC CTT GA), 0.05µM molecular beacon for the wild-type HIV-1 sequence (MB045: FAM-CGA CGT AGT AGT GTG TGC CCG TCT GTA CGT CG-dabcyl), 0.05µM molecular beacon for the system control RNA (MB054: ROX-CCG ACT CTC TAC ACA CCA GAC AAA AAA CGA GTC GG-dabcyl), 375mM sorbitol, 0.105 µg/µl bovine serum albumin, 6.4 units AMV RT, 32 units T7 RNA polymerase, 0.08 units RNase H and input nucleic acid. The complete mixture, except the enzymes was, prior to adding the enzymes, heated to 65°C in order to denature any secondary structure in the RNA and to allow the primers to anneal. After cooling the mixture to 41°C the enzymes were added. The amplification took place at 41°C for 60 min in a thermostated fluorimeter (CytoFluor 2000 or EasyQ Reader) and the fluorescent signal of the molecular beacon probe was measured every minute.

To achieve quantification, a dilution series of target sequence for a particular primer set was amplified and the time points at which the reactions became positive (the time to positivity, TTP) were plotted against the input amounts of nucleic acid. This way a calibration curve was created that could be used to read TTP values of reactions with unknown amounts of input and deduce the input amount.

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The results of the determinations of the same samples spotted and added directly to the lysis buffer were compared and the analysis is shown in figure 1 below. By the results of the system control RNA it appeared that there were no false negative results and all negative data reported in figure 1 are true negative data resulting from the absence of HIV-1 sequence or presence at concentrations below the detection limit of the tests.

Example 3

Plasma of 88 HIV-1 infected individuals was spotted in 200 µl droplets on S&S 903 paper (Schleiger & Schull) and dried in the air and stored for a minimum of 24 hours at ambient temperature. Simultaneously, 200 µl of the same plasma samples was directly added to the lysis buffer as described by Boom et al. (1990). The spots on the filter paper were pinched out and administered to a tube containing 4 ml lysis buffer as described by Boom et al. (1990). The tubes were mildly shaken on a shaking platform for 3 hours at ambient temperature. During this incubation the dried spot dissolves from the filter paper into the lysis buffer. Subsequently the filters were removed from the tubes with a cleaned pair of tweezers. Between tubes the tweezers were subsequently cleaned with hot water-chlorine-hot water-70% alcohol.

The nucleic acid now present in the lysis buffer was further purified with the method described by Boom et al (1990) or with dedicated isolation kits purchased from Qiagen (Qiagen GmbH, Max Volmer Strasse 4, 40724 Hilden, Germany) or Biomerieux (formerly Organon Teknika, Boseind 15, 5281 RM Boxtel, The Netherlands) and used according to the manufacturer's protocols. The isolated nucleic acid was stored at -80°C until further analysis. Usually 5 µl was used as input in NASBA amplification reactions determining the amount of HIV-1 RNA as described by De Baar et al [2,3].

Standard NASBA nucleic acid amplification reactions were performed in a 20µl reaction volume and contained: 40mM Tris-pH 8.5, 70mM KCl, 12mM MgCl2, 5mM dithiotreitol, 1mM dNTP's (each), 2mM rNTP's (each), 0.2µM primer (each), 0.05µM molecular beacon for the wild-type HIV-1 sequence, 0.05µM molecular beacon for the system control RNA, 375mM sorbitol, 0.105 µg/µl bovine serum albumin, 6.4 units AMV RT, 32 units T7 RNA polymerase, 0.08 units RNase H and input nucleic acid. The complete mixture, except the enzymes was, prior to adding the enzymes, heated to 65°C in order to denature any secondary structure in the RNA and to allow the primers to anneal. After cooling the mixture to 41°C the enzymes were added. The amplification took place at 41°C for 90 min in a thermostated fluorimeter (CytoFluor 2000 or EasyQ Reader) and the fluorescent signal of the molecular beacon probe was measured every minute.

To achieve quantification, a dilution series of target sequence for a particular primer set was amplified and the time points at which the reactions became positive (the time to positivity, TTP) were plotted against the input amounts of nucleic acid. This way a calibration curve was created that could be used to read TTP values of reactions with unknown amounts of input and deduce the input amount.

The results of the determinations of the same samples spotted and added directly to the lysis buffer were compared and the analysis is shown in figure 2.

The data in figure 2 clearly indicate a very good correlation between the results obtained with the direct admission of sample to the lysis buffer compared to first spotting of the sample on paper, drying and thereafter admission to the lysis buffer. When analyzed with the Pearson correlation test a correlation coefficient (r) of 0.919 for plasma direct and 0.959 for dried plasma was found.

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Figure legends

Figure 1. Comparison of quantitative HIV-1 data obtained on mother milk samples that were analyzed directly or were first spotted and dried on filter paper. The assay cut of is at log2, indicated by solid lines in the graph. The numbers on the axis indicates the log copy number of HIV-1 RNA molecules found in the test.

The data in figure 1 clearly indicate a very good correlation between the results obtained with the direct admission of sample to the lysis buffer compared to first spotting of the sample on paper, drying and thereafter admission to the lysis buffer.

Figure 2. Comparison of quantitative HIV-1 data obtained on plasma samples that were analyzed directly or were first spotted and dried on filter paper. The assay lower limit of detection is at log2, indicated by solid lines in the graph. The numbers on the axis indicates the log copy number of HIV-1 RNA molecules found in the test.

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Claims

1. A method for quantifying a nucleic acid of interest in at least one sample, comprising:

- administering said sample to a solid carrier capable of at least in part absorbing said sample;
- drying said carrier;
- providing at least part of said carrier comprising at least 100 µl of sample to a nucleic acid isolation solution so that nucleic acid is extracted from said carrier; and
- quantifying said nucleic acid of interest.

2. A method according to claim 1, wherein at least 250 µl of sample is analysed.

3. A method according to anyone of claims 1-2, comprising identifying said nucleic acid.

4. A method according to anyone of claims 1-3, wherein said solid carrier is provided with at least two samples.

5. A method according to anyone of claims 1-4, comprising administering to said solid carrier a known amount of a reference nucleic acid.

6. A method according to anyone of claims 1-5, wherein said part comprises the whole of said at least one sample.

7. A method according to anyone of claims 1-6, wherein said part comprises the whole of said solid carrier.

8. A method according to claim 4 or 5, wherein said part comprises one of said samples.

9. A method according to anyone of claims 1-8, wherein said nucleic acid isolation solution comprises a chaotropic nucleic acid isolation lysis buffer.

10. A method according to any one of claims 1-9, wherein said nucleic acid comprises RNA.

11. A method according to claim 10 wherein said RNA comprises mitochondrial RNA, viral RNA and/or messenger RNA.

12. A method according to anyone of claims 1-11, wherein viral nucleic acid is detected.

13. A method according to claim 12, wherein said viral nucleic acid comprises a retroviral nucleic acid.

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14. A method according to claim 11 or 12, wherein said viral nucleic acid comprises HIV and/or HTLV.

15. A method according to anyone of claims 11-14, wherein said viral nucleic acid comprises HIV-1.

16. A method according to anyone of claims 1-15, wherein said carrier comprises a filter-paper.

17. A method according to anyone of claim 1-16, comprising genotyping a mutant.

18. A method according to anyone of claims 1-17, wherein said sample comprises a precious body fluid.

19. A method according to anyone of claims 1-18, wherein said sample comprises blood, plasma, mothers milk, sputum, liquor, saliva, and/or urine.

20. A method according to anyone of claims 1-19, wherein said sample comprises a droplet of whole blood from a finger or heel puncture.

21. A method according to anyone of claims 1-19, wherein said sample is a plasma sample.

22. A method according to anyone of claims 1-21, wherein said nucleic acid detection and/or quantification comprises an amplification step.

23. A method according to claim 22, wherein said amplification comprises real-time monitored amplification.

24. A method according to anyone of claims 1-23, wherein said nucleic acid detection and/or quantification is performed with an end-point read-out system.

25. A method according to any one of claims 1-24 wherein a ratio between different nucleic acids is determined.

26. Use of a dried solid carrier provided with a sample for quantifying a nucleic acid of interest in said sample, wherein the nucleic acid present in at least the equivalent of 100 µl of blood or a derivative thereof is quantified.