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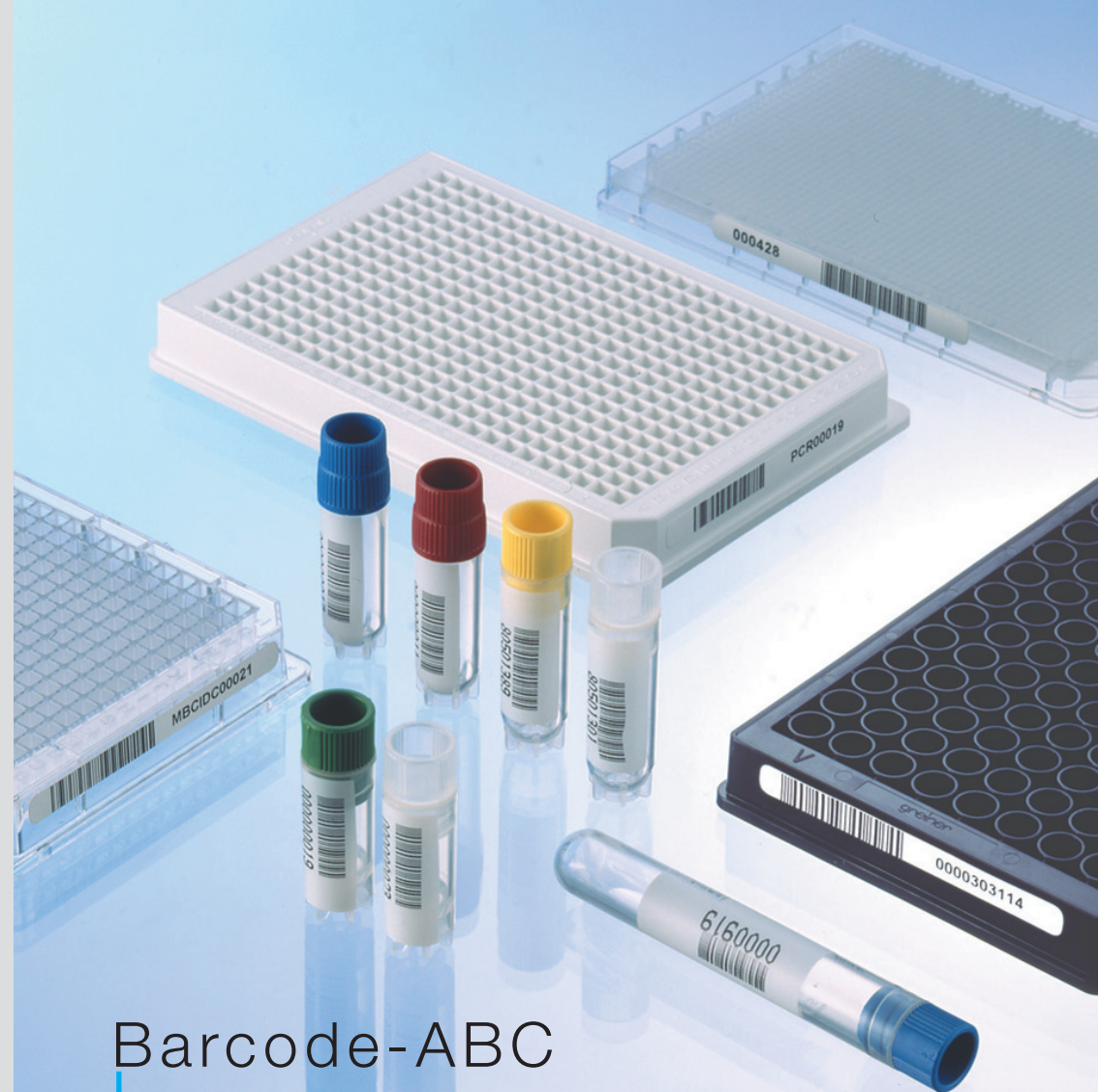
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Barcode-ABC

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1. History of the barcode!

The idea to use barcodes for the reliable and fast entering of data was developed soon after the invention of the computer. In the 1930's the first patents for barcode-like systems were registered and attempts towards the rationalisation of warehouse stocks using a barcode-like system were made. Due to the lack of suitable opto-electronic readers for scanners the development of barcodes could not be seriously pursued until after World War II. An initial patent for a round code was registered in the USA in 1949. The big breakthrough for barcode systems finally came in the 1960's and 70's thanks to various efforts to introduce unified systems and standards (1972, introduction of the Codabar; 1973, introduction of the Universal Product Code (= U.P.C.); 1974, introduction of Code 39, the first industrial barcode; 1976, establishment of the European Article Number Code (= EAN-Code) in Europe).

2. Why barcodes?

Originally arising from the logistics department of large supermarket chains, today barcode systems have won a place in many areas of everyday life.

Barcodes allow a reliable identification of samples or goods, are simple and universally applicable, and thus facilitate a smooth transfer of information.

In the biomedical sciences industry barcodes can be used for the identification of biological specimen materials, such as blood samples at blood banks, filling/storage of DNA samples and tissue samples in laboratory medical practices, and in the field of high throughput screenings in the identification and sorting of active ingredients and test results.

3. What is a barcode?

„A barcode contains information, which is encoded according to specific conventions (symbology) and graphically presents this information within the barcode field in the form of coloured stripes or bars and colourless spaces“. Normally a barcode contains no descriptive data, instead consisting of a different number of numbers or characters, depending on the type of barcode, which make up a reference number. With the aid of the reference number, information deposited in the form of a data set can be classified and retrieved. In order to handle numbers or characters in an electronic data processing, these must be brought into a form which is understandable for the machine, in other words in machine readable form. For a barcode, the specific information is encoded in the width of the bar, i.e. in the time needed to read it. The aim

of the encodement is always to achieve the maximal reliability and distinguishability of the characters.

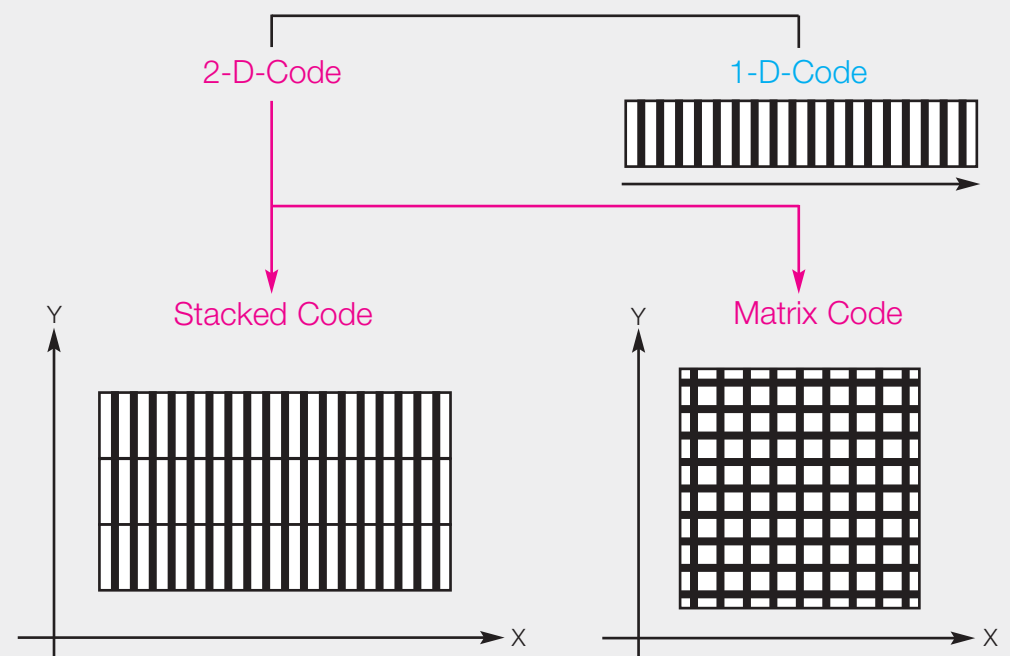
4. How does a barcode function?

The barcode is read by an optical reader. For 1-D barcodes this is usually a scanner, for 2-D barcodes generally a camera system. Thereby the light source of the

scanner is absorbed by the dark bars, while the light spaces are reflected. The scanner produces a weak electrical signal for the spaces and a strong signal for the bars. The duration of the signal is determined by how broad or narrow the individual elements are. This signal is converted by an integrated or an external decoder in a temporal sequence and transmitted to a computer.

5. Classification of barcodes

Basically one distinguishes between one dimensional and the new two dimensional barcodes introduced a few years ago.



5.1. One dimensional barcodes (1-D Barcode)

One dimensional barcodes consist of a line with coloured bars and colourless spaces and can be subclassified into two width and multiple width codes

Two width barcodes

The characters of a two width code consist of elements with two different widths, whereby V , the ratio between the wide and narrow elements lies between 2:1 and 3:1. That means that the wide elements are two to three times as wide as the narrow elements. Two width codes are easy to produce and place minimal requirements on the necessary reading equipment with regard to reading accuracy. The disadvantage of two width codes is their low information density. Hence they are only recommended for applications involving small amounts of information.

Two width codes include among others the **codes** of the family **2/5** or **Code 39**.

Multiple width codes

For multiple width codes the barcodes consist of elements with more than two widths.

Multiple width codes have a significantly higher information density than two width codes. This higher information density is accompanied by a greater demand on the print quality of the barcode and the precision

of the reading equipment. To avoid reading errors substantially narrower tolerances for the individual elements (bars and spaces) must be held to in the production of multiple width codes than in making a two width code. Multiple width codes include among others Code **EAN-13** (European Article Number) and its American counterpart, the **UPC-A** (Universal Product Code), as well as **Code 128**.

5.2. Two dimensional barcodes

The introduction of two dimensional barcodes in the 1990's allowed the representation of even larger amounts of data in the form of a barcode. Two dimensional barcodes fall into two categories, stacked code and matrix code.

The basic idea of a stacked code is the linking of individual 1-D codes, in that several barcodes are arranged one over the other. A stacked code consists of multiple rows of bars and spaces, usually with a common start and stop symbol. The matrix code in contrast consists of polygons, mostly rectangularly arrayed groups of data cells with typical orientation characters, from which the particular code can be recognised.

6. The most important basic terms for barcodes

6.1. The most important basic terms for barcodes

■ **Bar or stripe:**

The black element of a bar code.

■ **Space:**

This usually refers to the space between bars within a character. Gap is used to refer to the open background between the last bar of a barcode-character and the first bar of the next character.

■ **Element:**

Term to describe a bar or a space.

■ **Module:**

The narrowest element in a barcode is termed a module. Wide bars or spaces are calculated in multiples of the module.

■ **Module width X:**

Gives the width of the narrowest element.

■ **Discrete codes:**

Discrete codes are barcodes, for which every character begins and ends with a bar. Spaces contain no information and are not part of the code. Since every character begins and ends with a bar, the gaps between characters may vary within certain limits.

■ **Continuous codes:**

In contrast to a discrete code, for continuous barcode-types the internal spaces within the characters contain information (e.g. the intra-character spaces in Barcode 2/5 Interleaved).

■ **Reading error:**

The decoding of the barcode is not possible. The reason may be for example a defective detection system or poor printing quality.

■ **Substitution error:**

The barcode can be read but an incorrect value is read, e.g. due to unclear printing.

■ **Quiet zone:**

The white zone leading and trailing the 1-D barcode symbol. The quiet zone is necessary to set the reading direction to the orientation of the bar code. Generally for the quiet zone there is a minimum of $10x$ the module width X but at least 2.5 mm. For scanner applications with a large depth of focus a larger quiet zone must be chosen. Here the rule is $Q=15x$ module width X , but at least 6.5 mm.

■ **Coding field:**

The coding field consists of a barcode, two light quiet zones and a human readable or clear-text line. The barcode contains the encoded information, consisting of coloured bars and non-coloured spaces. A quiet zone is found leading and trailing the barcode and serves to delineate the boundaries of the object to be identified. The clear-text line is usually found beneath (for microplate labels usually beside) the barcode and presents the entire encoded information in human readable text.

■ **Check character:**

The check character serves for the detection of errors in reading a barcode (recognition of decoding substitution errors). In some barcodes, such as e.g. EAN 13, Code 128 and UPC-A, check characters are mandatorily required and cannot be optionally omitted, as is the case with Code 2/5 I or Code 39. **As a matter of principle the use of check characters is advised.** The check character is calculated according to certain rules (mathematical algorithms).

a check is made whether the printed check character agrees with the check character calculated from the barcode sequence. This digit is generally not carried over to the clear-text line, but it may be upon appropriate programming of the scanner.

Example:

The calculation to obtain the check character according to the method Modulo 10, with the weighting factors 3,1,3,1, ... (used for barcodes of the family 2/5 and EAN/UPC). The weighting factors are distributed from right to left.

Clear text	4 0 2 2
Check character	2
Data digit sequence	4 0 2
Weighting factors	3 1 3
Individual products	12 + 0 + 6 = 18
Modulo 10	18 : 10 = 1, remainder 8
Difference to 10 = Check character	10 - 8 = 2
Check character	2

The check character is also encoded in bars and spaces, placed immediately before the stop character of the barcode, and is thus a part of the barcode. In reading the barcode

■ **Start and stop characters:**

Start and stop characters delimit a barcode symbol. At the same time they also serve to permit bidirectional reading of a barcode. The start and stop characters of the barcode are usually constructed so that when read from left to right they have a different sequence than when read from right to left. In the barcodes EAN and UPC and the internationally used barcodes so called edge characters are used instead of the usual start and stop characters.

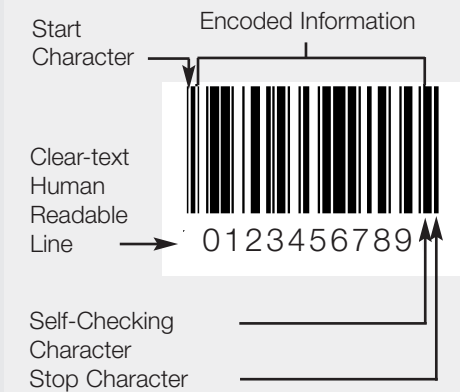
narrow. Now if the reader detects more or less than two wide elements (e.g. due to a printing defect) the barcode will not be recognised.

■ **Arrangement of a barcode**

(For an explanation of terms see terminology section above). The barcode field includes two quiet zones, one leading and one trailing the barcode, as well as a clear-text human readable line. In microplate-barcode labels this line may be found under or beside the actual barcode. The start and stop characters define the ends of the barcode. The self check digit serves to detect reading errors during the scanning of a barcode, and is not shown in the clear-text line.

■ **Self checking-barcode:**

The width ratio of a narrow to a wide element is 1:2 or 1:3. This ratio is monitored during reading and provides for quite good reading reliability. Most barcodes have however an additional self check. In many barcodes the individual characters of a barcode are always constructed according to a particular scheme. During reading a check is made if the prespecified arrangement is followed. For example, in Barcode 2/5 Interleaved the characters always have 5 elements per encoded digit. Of these, two elements are always wide and three elements

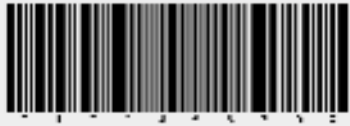


6.2. The most important one-dimensional barcode types

Code 2/5 (Industrial Two of Five)

Features:

- **Numerical code** (single-digit numbers from 0-9 representable).
- The code is based on two wide and three narrow bars per character (two width barcode).
- **The spaces contain no information.**
- The width ratio of a narrow bar to a wide bar is 1:2 to 1:3.



Example: Code 2 of 5

Advantages:

- The code consists only of bars, in the spaces there is no information encoded. Thus, **large tolerances of +/- 15%** are possible and the code is producible with **very simple printing processes.**

Disadvantages:

- **Low information density.**
- **High space requirement.** With a module width $X = 0.3$ mm and a width ratio $V = 1:3$, the code requires 4.2 mm for the representation of a single-digit number.

Code ITF (Interleaved Two of Five, Code 2/5 Interleaved)

Features:

- **Numerical code** (numbers from 0-9 representable).
- The code for a character consists of two wide and three narrow bars, or two wide and three narrow spaces (two width barcode).
- The first numerical character of a barcode is represented by 5 bars, the second by the spaces immediately following each of the bars of the first number, etc. **(So the spaces also contain information)**
- The width ratio (V) between a narrow element and a wide element must be at least 1:2, and maximally 1:3. If a narrow element is smaller than 0.5 mm, then a ratio V of narrow element to wide element of 1:1.25 up to a maximum of 1:3 is allowed.
- **Only number pairs are representable.**



0123456789

Example: Code 2 of 5 interleaved

Advantages:

- **High information density.** For example with a module width (X) of 0.3 mm and a width ratio $V = 1:3$, a digit can be represented in 2.7 mm
- **Barcode is self checking.**

Disadvantages:

- **Only number pairs** (only even numbers of bar code digits are allowed).
- The advantage of the high information density represents at the same time the disadvantage. Since all spaces carry information, tight tolerances must be maintained in printing. **Deviations of maximally 10 % can be tolerated.**
- Through the small start and stop characters there is a danger that truncated readings (partial scans) will not be recognised as such **(Barcode with limited reading reliability)**

Codabar

Features:

- In addition to the **single-digit numerical characters (0-9)**, **six special symbols/characters** (-, \$, :, /, ., +) can be represented.
- Each character is made up of 7 elements (4 bars and 3 spaces). Either two or three wide and four or five narrow **elements are used. The spaces between the characters carry no information.**

- The allowable ratio of the width of a narrow element to a wide element is from $V = 1:2.25$ to maximally $V = 1:3$.
- The Codabar is widely used in the **medical-clinical field.**



Example: Codabar

Advantages:

- The advantage of the Codabar is the possibility for the **representation of six special symbols.**

Disadvantages:

- The incomplete utilisation of the spaces results in a **low information density.** Thus for example, with a module width (X) of 0.3 mm and a width ratio $V = 1:3$, 5.5 mm are needed to represent a digit.

Code 39 (Code 3 of 9)

Advantages:

- **Alphanumerical code;** besides the **numerical characters (0-9)**, **26 letters** as well as **seven special symbols** can be represented.
- Each character consists of 9 elements (five bars and four spaces). Three of the

nine elements are wide and six are narrow (Two width barcode).

- The **space between characters has no information**. The allowable width ratio of a narrow element to a wide element ranges from 1:2 up to maximally 1:3.
- If the narrow element is smaller than 0.5 mm, then the allowable ratio of a narrow element to a wide element is from 1:2.25 up to maximally 1:3.
- The **module width X should be at least 0.2 mm**.



* 1 2 3 4 5 6 7 *

Example: Code 39

Advantages:

- **Representation of alphanumerical characters.**
- **No stringent demands** on the printing technique.
- **High reading reliability** with the inclusion of a check character (1:2.5 Mio).
- **Readable under extreme conditions** (large distances).

Disadvantages:

- **Low information density**; To represent a single digit, at a module width $X = 0.3$ mm and an element width ratio of 1:3, one requires 4.8 mm.
- **Close tolerance** of maximally 10%.

Code 128

Features:

- Code 128 permits, without any need to use character combinations, representation of the **full ASCII-character set**.
- In Code 128 three character sets are distinguished (**128 A, 128 B, 128 C**) and depending on the problem presented, their **intermixing is possible**.
- In order to be able to **represent the full ASCII-character set, one needs the start character A or B** in combination with one of the special symbol characters of Code 128.
- Code **128 C cannot code letters** and must have an even number of places since **only digit pairs** can be represented.
- **128 B can code letters as well as numbers.**
- **128 A can code special symbols.**
- Each character consists of 11 modules, distributed over three bars and three spaces. The bars are always made up of an even number of modules (even parity) and the spaces of an odd number of modules.
- The stop character represents an exception and comprises 13 modules, consisting of 11 modules and an end bar with two modules.



Code 128

Example: Code 128

Advantages:

- Allows the **representation of the full ASCII-character set**.
- **High information density**.
- **High reading reliability**.
- **Space saving**. Representation of digit pairs on half the area by **numerical compression (128C)**.

Disadvantages:

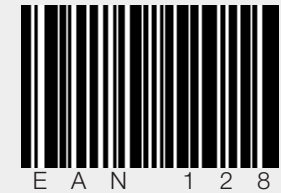
- **Strict tolerance** (Code 128 is a four width code).
- For a constant data length **no constant code length**.
- **ASCII-character set not completely representable with a character set of barcode 128 A or B, but automatic switchover to character set C is usual.**

EAN 128 (European Article Numbering)

Features / Advantages / Disadvantages:

- EAN 128 is a **logistics code for trade**. It corresponds to Code 128, except that as a start character the combination of Start A, Start B or Start C with the special character FNC1 is used.

- The physical length of an EAN 128 barcode **must not exceed 165 mm**.
- Including an initial application indicator (AI), a **maximum of 48 data characters may be coded**.
- Including all auxiliary and check characters an EAN 128 barcode **symbol may not exceed a length of 35 characters**.



E A N 1 2 8

Example: EAN 128

EAN (EAN-8/EAN-13)

Features:

- **Numerical code**; Digits 0-9 can be represented.
- Each character is made up of 11 elements.
- All bars and **spaces carry information**.
- **Only 8 or 13 digits** can be represented (EAN-8, EAN-13).
- This barcode is primarily used in **trade**.

Advantages:

- **high information density** in 10 different sizes.

Disadvantages:

- Very **close tolerances**.
- **Unreliable** (Reliability of 1:1000, **unsuitable for medical applications**).

the character sets A and B are used. Thus only the 10 digits can be represented with UPC-E. The inclusion of a check character is specified.



UPC (Universal Product Code UPC-A / UPC-E)

Features:

- American counterpart of the EAN Code.
- Two UPC versions are standard (UPC-A and UPC-E).
- UPC-A is the version of the barcode commonly used.
- Version E is an abridged version for applications where limited space is available.
- **UPC-A is comparable to EAN 13**, except that only 12 instead of 13 places are used. Consequently the two barcode types EAN 13 and UPC-A are not compatible with one another. The character set of UPC-A comprises 10 digits from two character sets (A and C), whereby a start, a stop and a separation character are used.
- UPC-E is set up for 8 places, whereby

6.3. Overview of the most important barcodes:

Barcode	UPC-A UPC-E	EAN 8 EAN 13	2/5 Interleaved	Code 39	Code 128
Type	Numerical	Numerical	Numerical	Alphanumerical	Alphanumerical
Character set	10 digits (0-9)	10 digits (0-9)	10 digits (0-9)	10 digits, 26 letters, 7 special symbols	128 ASCII (Full ASCII)
Check digit	yes	yes	yes optional	yes optional	yes
Variable character length	no (6,12)	no (8,13)	Limited, only even numbers of digits	yes	yes
Distribution	USA/Canada	Europe /worldwide	worldwide	worldwide	worldwide
Primary usage	Commerce	Commerce	Materials handling technology	Universal (Industry Commerce, Transport, Medical Administration)	Universal (Industry Commerce, Transport, Medical Administration)
Microplates	no	no	yes	yes	yes
Cryo.s™	no	no	yes	yes	yes

6.4. Criteria for the selection of a suitable 1-D barcode:

All the one dimensional barcodes described in this brochure can be used for microplates and Cryo.s™ without any problems. Code 39 and Code 128C have widespread application.

Generally, in selecting a suitable barcode, fast and reliable data acquisition should be the primary concern. That means:

- for 4-10 digits **the shortest possible barcode** should be used, e.g. Code 39; for 8-20 digits, use for example Code 2/5 interleaved.
- **the simplest possible barcodes** should be used (for example 2/5 Family, Code 39, barcodes with only two different space and bar widths).
- **module width** should be matched to the resolution limits of the reading equipment.
- for a reliable reading of the barcode attention should be paid to having a **reliable print quality**.
- **the information density** (CPI= characters per inch) should not be too high, and thereby the module width not too low. Based on good previous experience, we have selected a module width $X = 0.5$ mm, when no other value is noted in the barcode-ordering format.

Information density	Module width X
Ultra High Density-Code	$X < 0.19$ mm
High Density Code	$0,19$ mm $< X < 0.24$ mm
Medium Density Code	$0,24$ mm $< X < 0.30$ mm
Low Density Code	$0,30$ mm $< X < 0.50$ mm
Use at greater distances	$X > 0.50$ mm

- **Barcode height:** A barcode should be at least 6.5 mm or 15% of the total length of the code (This rule also holds for scanning pens.)

- **Combination possibilities with colour printing:** Not every conceivable colour combination with respect to barcode and background colour is recommended for barcodes. For example for the reading of barcodes with red coloured bars a detection system with blue light illumination is necessary. It should be kept in mind, that the basic prerequisite for reliable and rapid

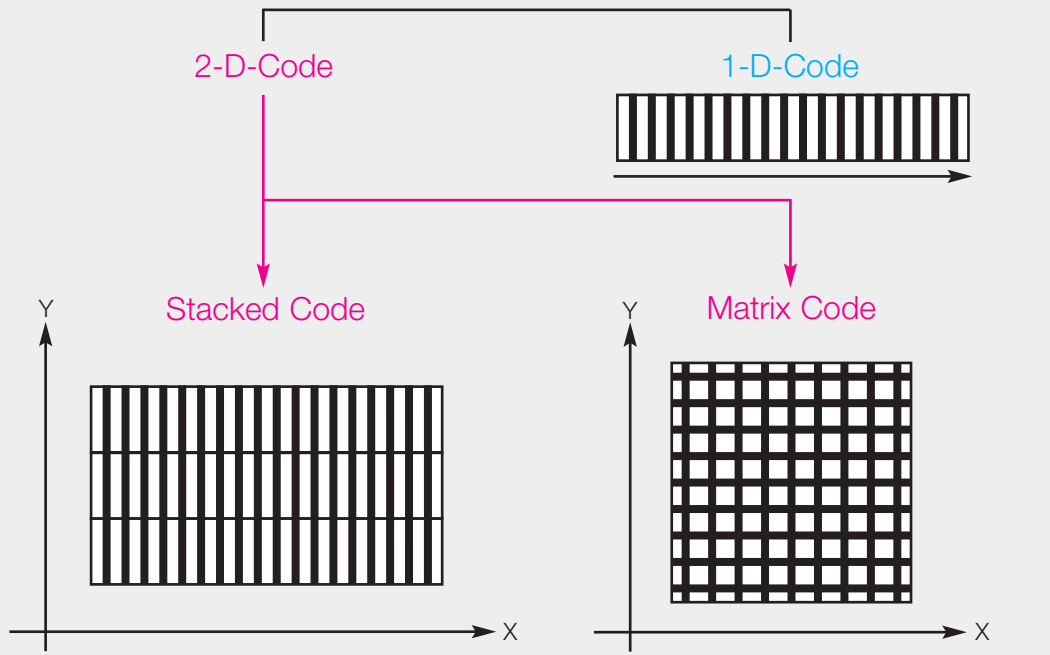
reading is a high barcode quality with respect to printing and contrast. Both criteria are best fulfilled by the choice of a black bar colour on a white background. Cryo.s™ are offered by Greiner Bio-One exclusively in black bar colour on a white background, because of the direct printing process used. For labelling of microplates, coloured printing is available upon request.

- Both the barcodes on Cryo.s™ as well as barcodes on microplates are carefully monitored within the framework of our internal quality controls.

7. Two dimensional barcodes

After one dimensional barcodes became successfully established in the 1970's and 80's in numerous application areas, the demand arose for barcodes, which were able to encode larger amounts of data error-free. This demand was met with the development of two dimensional barcodes.

Two dimensional barcodes fall into two different classes: the class of stacked barcodes and the class of matrix codes.



The basic idea of a stacked code is the linking of individual 1-D codes, in that several barcodes are arranged one over the other. Hereby the actual information is encoded along the x-axis, and the row information in the y-axis.

The advantages of stacked codes **include the minimal additional effort in going from**

1-D to 2-D, since the same printing and evaluation systems can be used.

Furthermore, it is possible to build on the existing code families and their decoding routines, whereby the incorporation into existing applications is further simplified. In contrast to this, with the matrix 2-D barcodes a completely different kind of 2-D

barcode has been developed which has very little in common with the classical barcode.

Matrix codes are as a rule read with **cameras** and other **image processing systems**.

Processing (= recognition) of the barcode is possible in any arbitrary position of the code. Matrix codes must however be equipped with **fixed code elements for the recognition of position** for the scanner. The advantage of all matrix codes is the high information density and low space requirement. In the summary presented

below emphasis is placed on the two most important matrix codes (DataMatrix and Maxicode). There are however other matrix codes available on the market.

At present Greiner Bio-One does not yet offer two dimensional barcodes as a standard product. Cryo.s™ and micro-plates can however be equipped with matrix code upon request. If you are interested in having other products outfitted with two dimensional barcodes, do not hesitate to contact us (info@de.gbo.com).

7.1. The most important two-dimensional barcode types

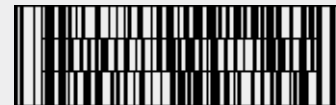
7.1.1. Stacked codes

Stacked code 49

- Code 49 is a variant of the stacked barcode, for which the number of stacked rows can vary from two to eight.

Codablock F

- Codablock F is a stacked variant of the standard barcodes 39 and 128 with linkage of the rows.
- up to 44 rows with two to 62 characters; maximally 2725 characters can be generated.



Example: Codablock F

Code 16 K

- Code 16 K is a variant of the stacked barcode based on elements of the UPC and Code 128.
- The number of rows can vary between 2 and 16.
- Each row is recognised by a start and stop character and has two check digits.



Example: Code 16 K

PDF 417 (Portable Data File 417)

- PDF 417 is a variant of the stacked barcode based on its own code

structure, consisting of its own code words.

- Characters are encoded in "code words", whereby each code word is made up of 17 modules, each containing 4 bars and 4 spaces.
- The number of rows can vary between 3 and 90.
- Up to 1000 characters can be coded.
- Different error correction levels can be programmed.



Example: PDF 417

7.1.2. Matrix code

DataMatrix

Features

- The DataMatrix Code has a variable rectangular size in the form of a matrix.
- The matrix consists of at least one square array made up of at least 10x10 and maximally 244x144 symbol elements.
- In the maximal size 2334 ASCII-characters (7 bit) or 1558 extended ASCII-characters (8 bit) or 3116 digits can be encoded. A solid bar L frames two adjacent sides as a finder for orientation of reading by a detection system. If this frame is damaged, the

matrix code cannot be recognised. The information density is 13 characters per 100 mm².

- There are two versions of the DataMatrix Code available: Version A ECC 000-140 and Version ECC 200. Version ECC 200 is a more up-to-date revision of DataMatrix.



Example: DataMatrix

Advantages:

- Very compact code.
- Very reliable, as it has a powerful error-correction algorithm (Reed Solomon) built in.
- Reconstruction of the data content even after damage to the total code symbol of up to 25%.
- Omnidirectional readability also at high transport speeds.

Disadvantage:

- Readable only with image processing systems (Image Reader / 2D scanners).

HIBC (Health Industry Care Bar Code)

Currently two dimensional HIBC barcodes are primarily used in medical areas for example for labelling operating instruments, in laboratory diagnostics for the identification

of patient material, as well as by blood banks for the labelling of blood supplies. The goal in all applications is an unequivocal sorting of the sample materials by means of an unequivocal barcode. The HIBC makes possible a nomenclature that assigns each labelled piece a unique barcode number. Overlapping or multiple assignment of the same number, as is possible with 1-D barcodes, cannot occur.

Maxicode

The Maxicode is a variant of the matrix code. It has a fixed size of 25.4 mm x 25.4 mm. On an area of 654 mm² 144 symbols can be represented, or maximally 93 ASCII characters or 138 digits. In the middle there is always a search pattern consisting of three concentric circles, which serves to orient the reading process. Around this search pattern are 866 hexagons, arranged like a honeycomb in 33 rows, which contain the data.

Advantages:

- Very compact code.
- Very reliable, as it has a powerful built-in error-correction algorithm.



Example: Maxicode

- Reconstruction of the data content after damage to the total code symbol of up to 25%.
- Omnidirectional readability also at high transport speeds.

Disadvantage:

- Fixed parameters.
- Readable only with image processing systems (Image Reader / 2D scanners).

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Additional information sources used were:

barcode-ABC, Datalogic GmbH Uracher Strasse 22, D-73268 Erkenbrechtsweiler
The Barcode Book, Roger C. Palmer, Helmers Publishing Inc. 174 Concord Street, Peterborough, New Hampshire